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(54) Title: ANTI-INFLAMMATORY COMPOUNDS			
(57) Abstract <p>This invention relates to the novel compounds and pharmaceutical compositions of formula (I) and of formula (II). This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of formula (I) or (II).</p>			

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ANTI-INFLAMMATORY COMPOUNDS

FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions and their use as anti-inflammatory agents in mammals.

BACKGROUND OF THE INVENTION

An early event in the response of most inflammatory cells to immunologic activation and other stimuli is the release of newly formed products (mediators) which alter the function and biochemistry of surrounding cells and tissues. The ensuing biological responses, as well as much of the pathogenesis which is attributed to inflammation and allergy, are thought to be dependent on the effects that these newly-formed mediators have on adjacent cells within the inflammatory region.

In the last 20 years, it has become apparent that lipid mediators are among the most potent and important products which are generated during inflammatory reactions. The synthesis of most lipid mediators is initiated by the specific cleavage of complex phospholipid molecules which contain arachidonate at their sn-2 position. Arachidonic acid is predominantly found in the sn-2 position of phospholipids after redistribution by transacylases and its release by sn-2 acylhydrolases from phospholipids represents the rate-limiting step in the formation of eicosanoids (leukotrienes, prostaglandins and thromboxanes) and other hydroxylated fatty acids. As arachidonic acid is released, it is then converted to oxygenated derivatives by at least two enzymatic systems (lipoxygenase and/or cyclooxygenase). Concomitant with arachidonate release, lysophospholipids are formed. One of these lyso phospholipids, 1-alkyl-2-lyso-sn-glycero-3-phosphocholine, is then acetylated to form platelet-activating factor (PAF). Each of the cell types involved in the inflammatory response produce and secrete a unique subset of lipid mediators. The quantities and nature of the metabolites depend on which enzymes and precursor phospholipid pools are available to inflammatory cells.

Once lipid mediators such as PAF and eicosanoids are formed by the aforementioned pathways, they induce signs and symptoms observed in the pathogenesis of various inflammatory disorders. Indeed, the pathophysiological activity of arachidonic acid (and its metabolites) is well known to those skilled in the art. For example, these mediators have been implicated as having an important role in allergy, asthma, anaphylaxis, adult

respiratory distress syndrome, reperfusion injury, inflammatory bowel disease, rheumatoid arthritis, endotoxic shock, and cardiovascular disease. Aalmon et al., Br. Med. Bull (1978) 43:285-296; Piper et al., Ann. NY Acad. Sci. (1991) 629:112-119; Holtzman, Am. Rev. Respir. Dis. (1991) 143:188-203; Snyder, Am. J. Physiol. Cell Physiol. (1990) 259:C697-C708; Prescott et al., J. Biol. Chem. (1990) 265:17381-17384.

Similar to arachidonate products, PAF is a potent proinflammatory mediator produced by a variety of cells. In vitro, PAF stimulates the movement and aggregation of neutrophils and the release therefrom of tissue-damaging enzymes and oxygen radicals. PAF has also been implicated in activation of leukocytes, monocytes, and macrophages. These activities contribute to the actions of PAF as having (pathological) physiological activity in inflammatory and allergic responses. PAF has also been implicated in smooth muscle contraction, pain, edema, hypotensive action, increases in vascular permeability, cardiovascular disorders, asthma, lung edema, endotoxin shock, and adult respiratory distress syndrome. PAF elicits these responses either directly through its own cellular receptor(s) or indirectly by inducing the synthesis of other mediators.

Accordingly, a method which antagonises the production of free arachidonic acid, its metabolites or PAF will have clinical utility in the treatment of a variety of allergic, inflammatory and hypersecretory conditions such as asthma, arthritis, rhinitis, bronchitis and urticaria, as well as reperfusion injury and other disease involving lipid mediators of inflammation. Many published patent applications or issued US patents exist which describe various compounds having utility as PAF or eicosanoid antagonists. Such patents include U.S. Pat. No. 4,788,205, 4,801,598, 4,981,860, 4,992,455, 4,983,592, 5,011,847, 5,019,581 and 5,002,941.

Phospholipase A₂'s (PLA₂ (EC 3.1.1.4)) are responsible for the liberation of arachidonic acid from the sn-2 position of phospholipid. They are thought to play an important role in the pathogenesis of inflammation and possibly in immunological dysfunction, both as a cell associated enzyme as well as an extracellular soluble enzyme. Low molecular weight, mammalian Type II 14 kDa PLA₂ has been well characterized and is known to exist in both an extracellular form in inflammatory fluids (Kramer et al., J. Biol. Chem., 264:5768-5775 (1989) and in a cell associated form (Kanda et al., Biochemical and Biophysical Research Communications, 163:42-48 (1989) and has been found in a variety of cells and tissues or extracellularly when released in response to antigenic activators or pro-inflammatory mediators such as Interleukin (IL)-1, IL-6 or tumor necrosis factor (TNF). Its presence in such inflammatory fluids, tissue exudates or serum has therefore implicated Type II-14 kDa-PLA₂'s role in inflammation (Vadas, et al., (1985) Life Sci. 36, 579-587; and Seilhamer, et al., (1989) J. Biol. Chem. 264, 5335-5338). Recently, the elevated serum levels of PLA₂ activity during an inflammatory insult has been attributed to

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cytokine induction of acute phase protein release from liver, of which the 14 kDa-PLA₂ is suggested to be a part (Crowl, et al., (1991) J. Biol. Chem. 266, 2647-2651). In addition, soluble PLA₂ activity is markedly elevated in the serum and synovial fluid of patients with rheumatoid arthritis (Stefanski et al., J. Biochem. 100:1297-303 (1986).

5 Furthermore, increasing serum PLA₂ levels have been shown to positively correlate with clinical severity (Bomalaski and Clark, Arthritis and Rheumat. 36:190-198 (1993)). Various inhibitors of PLA₂ have been described in publications and in US Patents. See for instance US Patents 4,959,357; 4,933,365; 5,208,223; 5,208,244; Marshall et al., J. Rheumatology 18:1 (1991); Marshall et al., Phospholipase A₂, Ed. Pyu Wong, Plenum
10 Press, NY (1990) pages 169-181; Wilkerson, et al., Eur. J. Med. Chem., 26:667, 1991 and Wilkerson, Antiinflammatory Phospholipase A₂ Inhibitors, Drugs of the Future, Vol. 15, No. 2 p 139-148(1990). Accordingly, as PLA₂ is important in the liberation of arachidonic acid from phospholipid and may also play a role in the generation of PAF via lysophospholipid formation, inhibition of such an enzyme would be useful for the treatment
15 of disease states caused thereby.

There are many novel forms of phospholipase A₂'s which have recently been discovered. For the purposes herein, members of the sn-2 acylhydrolase family of PLA₂'s are divided into low and high molecular weight enzymes be it from mammalian, or non-mammalian sources. Low molecular weight PLA₂'s will generally have a molecular weight
20 in the range of 12,000 to 15,000. High molecular weight will be in the range of 30,000 or 56,000 kDa to 110,000 by SDS electrophoresis analysis.

A high molecular weight, cytosolic 85 kDa PLA₂ has been isolated and cloned from the human monocytic cell line, U937 (Clark et al., Proc. Natl. Acad. Sci., 87:7708-7712, 1990). The cell-associated Type II-14 kDa-PLA₂ in cell lipid metabolism was thought to be
25 the key rate limiting enzyme in lipid mediator formation, until the recent identification of this cell-associated but structurally distinct 85 kDa sn-2 acylhydrolase, (Clark, et al., supra); and Kramer, et al., (1991) J. Biol. Chem. 266, 5268-5272. Like the Type II-14 kDa enzyme, this enzyme is active at neutral pH and Ca²⁺-dependent, but in contrast exhibits a preference for AA in the sn-2 position of phospholipid substrate and migrates from the cytosol to the
30 membrane in a Ca²⁺-dependent manner and is regulated by phosphorylation (Kramer et al., J. Biol. Chem., 266:5268-5272 (1991). The 85 kDa-PLA₂ is also distinct from 14 kDa-PLA₂s and Ca²⁺-independent PLA₂ as demonstrated by different biochemical characteristics such as stability of the 85 kDa-PLA₂ to DTT, instability to heat and the lack of inhibition by a phosphonate phospholipid TSA inhibitor of 14 kDa-PLA₂. In addition, 85 kDa-PLA₂ has
35 been shown to possess a lysophospholipase A₁ activity which is not observed with the 14 kDa-PLA₂s. The 85 kDa enzyme is similar to the myocardial Ca²⁺-independent PLA₂ (Bomalaski and Clark, Arthritis and Rheumat. 36:190-198 (1993)) in that Ca²⁺ is not

required for catalysis and DTNB inhibition is observed. However, 85 kDa-PLA₂ is not inhibited by the suicide inactivator bromoenol lactone, suggesting that the enzyme is distinct from the myocardial enzyme as well.

5 These characteristics make the 85 kDa-PLA₂ a candidate for participation in the liberation of AA from phospholipid stores for subsequent metabolism to lipid mediators. Both the cytosolic 85 kDa PLA₂ and a cell associated Type II 14 kDa PLA₂ have been found in the human monocyte, neutrophil and platelet (Marshall and Roshak, *Biochem. Cell Biol.* 71:331-339 (1993)). As noted above most of the cellular lipid mediators found
1 0 elevated in a variety of inflammatory fluids are formed in response to non-pancreatic 14 kDa PLA₂ action. Since arachidonate-containing phospholipids are the key precursors for a broad range of lipid mediators it would not be surprising that, inflammatory cells would treat these phospholipids differently than other fatty acid-containing phospholipids. In particular, there are enzymes which control the amount of arachidonate in different phospholipid pools and these enzymes are tightly regulated to maintain arachidonate
1 5 homeostasis. The movement of arachidonate into and from all phospholipids was originally thought to be exclusively by Coenzyme A-dependent acyl transferase activities. Holub *et al.*, *Adv. Lipid Res.*, 16:1-125 (1978); Lands *et al.*, In *The Enzymes of Biological Membranes*, ed. Martonosi, A., pp. 3-85, Plenum Press, NY, 1976. However, it has now been demonstrated that an enzyme, Coenzyme A-independent transacylase (CoA-IT), is
2 0 involved in the movement of 20 carbon higher unsaturated fatty acids, particularly arachidonate, into particular (1-alkyl- and 1-alkenyl) phospholipid pools. These are the phospholipid pools of arachidonate that are preferentially mobilized during cell activation and utilized for eicosanoid and PAF biosynthesis, respectively.

CoA-IT has a specificity for certain phospholipids as donor and acceptor molecules.
2 5 The fatty acid transferred is long chained and unsaturated, and almost exclusively arachidonate. Other fatty acids such as the 16:0, 18:1 or 18:2 are not moved into the sn-2 position of alkyl and 1-alkenyl phospholipid pools by CoA-IT. The specificity of CoA-IT is in direct contrast to many other CoA-dependent acylation activities which acylate a wide variety of lysophospholipids with no selectivity for arachidonate.

3 0 Accordingly, as CoA-IT is involved in arachidonic acid and phospholipid metabolism, inhibition of such an enzyme would be useful for the treatment of inflammatory, allergic and hypersecretory conditions or disease states caused thereby. Therefore, a method by which CoA-IT is inhibited will consequently and preferentially decrease the arachidonate content of 1-alkyl- and 1-alkenyl-linked phospholipids and will
3 5 therefore decrease the production of pro-inflammatory mediators such as free arachidonic acid, prostaglandins, leukotriene and PAF during an inflammatory response.

SUMMARY OF THE INVENTION

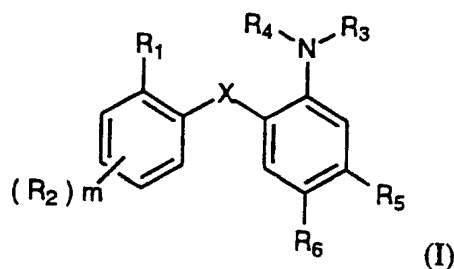
This invention relates to the novel compounds of Formula (I) and pharmaceutical compositions thereof which comprises a compound of Formula (I), or pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

- 5 This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of Formula (I).

- This invention also relates to a method of treating disease or disorders mediated by lipid inflammatory mediators, free arachidonic acid, its metabolites and/or PAF by administering to a patient in need thereof, an effective amount of a compound of Formula (I).

- 10 This invention also relates to a method of treating disease or disorders mediated by phospholipase A₂ (PLA₂) and/or Coenzyme A-independent transacylase (CoA-IT) by administering to a patient in need thereof, an effective amount of a compound or composition of Formula (I).

- 15 One aspect of the present invention are compounds having the structure represented by the formula:



wherein

- 20 R₁ is SO₃H or S(O)_nC₁₋₄ alkyl;
 n is 0 or an integer having a value of 1 or 2;
 R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;
 m is an integer having a value of 1 or 2;
 R₃ is -C(O)R₇ or -C(S)R₇;
 25 R₄ is hydrogen, or C₁₋₄ alkyl;
 R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₈, or (CH₂)_tOH;
 t is 0 or an integer having a value of 1 or 2;
 R₆ is hydrogen or halogen;
 R₇ is NR₉R₁₀;
 30 R₈ is hydrogen or C₁₋₄ alkyl;
 R₉ is hydrogen or C₁₋₄ alkyl;
 R₁₀ is optionally substituted aryl, optionally substituted arylC₁₋₂ alkyl, optionally

substituted C₁₋₈ alkyl, or R₉ and R₁₀ together with the nitrogen to which they are attached form a 5 to 7 membered saturated or unsaturated ring which may optionally comprise an additional heteroatom selected from O/N or sulfur;

X is oxygen or sulfur;

5 provided that

- a) when R₂ is 2,2-dimethyl propyl, m is 1, R₁ is SO₃H, R₅ is CF₃, and R₃ is C(O)NR₉R₁₀, and R₉ is hydrogen, than R₁₀ is other than 3,5-trimethyl phenyl; or
- b) when R₂ is 2,2-dimethyl propyl, m is 1, R₁ is SO₃H, R₅ is CF₃, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than 4-chloro-3-trifluoromethyl phenyl ;
- 1 0 c) when R₂ is 2,2-dimethyl propyl, m is 1, R₁ is SO₃H, R₅ is CF₃, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than 3-trifluoromethyl phenyl;
- d) when R₁ is SO₃H, R₂ is 5-methyl-4-chloro, m is 2, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 3-CF₃-4-
- 1 5 chlorophenyl, 3,4-dichlorophenyl, or 2-methyl-6-chlorophenyl;
- e) when R₁ is SO₃H, R₂ is 4,6-dichloro, m is 2, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 2-methyl-4-chlorophenyl;
- f) when R₁ is SO₃H, R₂ is 4-chloro, m is 1, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 2,5-dichlorophenyl, 3,5-di-
- 2 0 trifluoromethyl phenyl, 2-chloro-5-CF₃-phenyl, 3-CF₃-4-chlorophenyl, 3,4-dichlorophenyl, 2,3-dichlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 3-chloro-4-(4-chlorophenoxy), 3-CF₃-6-(4-chlorophenoxy), 3-chloro-6-(4-chlorophenoxy);
- 2 5 g) when R₁ is SO₃H, R₂ is 4,5-dichloro, m is 2, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 2-chloro-5-CF₃-phenyl or 3-CF₃-4-chlorophenyl ;
- h) when R₁ is SO₃H, R₂ is 4,5-dichloro, m is 2, R₅ is CF₃, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 4-chloro-3-CF₃phenyl;
- 3 0 i) when R₁ is SO₃H, X is sulfur, R₂ is 4,5-dichloro, m is 2, R₅ is trifluoromethyl, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;
- j) when R₁ is SO₃H, X is sulfur, R₂ is 4-chloro, m is 1, R₅ is trifluoromethyl, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a phenyl
- 3 5 substituted one to two times independently with chlorine or trifluoromethyl;
- k) when R₁ is SO₃H, X is sulfur, R₂ is 4-chloro, m is 1, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a phenyl

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substituted one to two times independently with chlorine or trifluoromethyl;
or a pharmaceutically acceptable salt thereof.

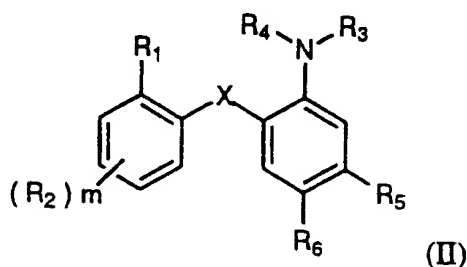
This invention also relates to the novel compounds of Formula (II) and
5 pharmaceutical compositions thereof which comprises a compound of Formula (II), or
pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

This invention also relates to a method of treating or reducing inflammation in a
mammal in need thereof, which comprises administering to said mammal an effective
amount of a compound or composition of Formula (II).

10 This invention also relates to a method of treating disease or disorders mediated by
free arachidonic acid, its metabolites and/or PAF by administering to a patient in need
thereof, an effective amount of a compound of Formula (II).

This invention also relates to a method of treating disease or disorders mediated by
phospholipase A₂ (PLA₂) and/or Coenzyme A-independent transacylase (CoA-IT) by
15 administering to a patient in need thereof, an effective amount of a compound or
composition of Formula (II).

One aspect of the present invention are compounds having the structure represented
by the formula:



wherein

- R₁ is SO₃H or S(O)_nC₁₋₄ alkyl;
n is 0 or an integer having a value of 1 or 2;
25 R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;
m is an integer having a value of 1 or 2;
R₃ is -C(O)R₇ or -C(S)R₇;
R₄ is hydrogen, or C₁₋₄ alkyl;
R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₈, or (CH₂)_tOH;
30 t is 0 or an integer having a value of 1 or 2;
R₆ is hydrogen or halogen;
R₇ is optionally substituted aryl, optionally substituted arylC₁₋₂ alkyl, or optionally

substituted C₁₋₈ alkyl ;
R₈ is hydrogen or C₁₋₄ alkyl;
X is oxygen or sulfur;
or a pharmaceutically acceptable salt thereof.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a novel method of treating inflammatory disease in a mammal in need thereof by administering to said mammal an effective amount of a compound according to Formula (I) or (II). The compounds of Formula (I) and (II) may
1 0 selectively inhibit the PLA₂ enzyme, the CoA-IT enzyme or both. Inhibition of either or both enzymes will result in the treatment of inflammatory occurrences in mammals. Inflammatory states in mammals may include, but are not limited to, allergic and asthmatic manifestations, dermatological diseases, inflammatory diseases, collagen diseases, reperfusion injury and stroke. Treatment of both acute and chronic diseases are possible.
1 5 Preferred diseases for treatment are arthritis, asthma, allergic rhinitis, inflammatory bowel disease (IBD), psoriasis, reperfusion injury and stroke. For the purposes herein, the compounds of Formula (I) and (II) are preferential and selective inhibitors of the low molecular weight PLA₂ enzyme.

2 0 For compounds of Formula (I) R₁ is suitably SO₃H or S(O)_nC₁₋₄ alkyl, and n is a number having a value of 0 to 2. Preferably R₁ is SO₃H or a salt thereof.

Suitably, R₂ is independently a substituent on the benzene ring from 0 to 2 times, and such substituent is suitably selected from hydrogen, halogen, an optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy group. Suitably when R₂ is halogen it is a chlorine or bromine.
2 5 When R₂ is an optionally substituted C₁₋₈ alkyl, the alkyl is substituted one to three times with halogen, such as fluorine, preferably a trifluoromethyl group. The optionally substituted C₁₋₈ alkyl moiety is preferably a branched C₅ chain, such as 1,1-dimethyl propyl moiety or a C₈ branched chain such as 1,1,3,3-tetramethyl butyl moiety. Suitably m is an integer having a value of 1 or 2. Most preferably R₂ is hydrogen.

3 0 Suitably, R₃ is C(O)R₇ or -C(S)R₇ and R₇ is suitably optionally substituted aryl, an optionally substituted arylC₁₋₂ alkyl, an optionally substituted C₁₋₈ alkyl group or the group NR₉R₁₀. Suitably R₉ is hydrogen or C₁₋₄ alkyl; and R₁₀ is hydrogen, optionally substituted aryl, optionally substituted arylC₁₋₂ alkyl, optionally substituted C₁₋₈ alkyl, or together R₉ and R₁₀ with the nitrogen to which they are attached form a 5 to 7 membered
3 5 saturated or unsaturated ring which may optionally comprise an additional heteroatom selected from O/N or sulfur.

Suitably the aryl, aryl alkyl or alkyl moieties of R₇ and R₉ and R₁₀ are substituted

independently, one to three times, by halogen, trifluoromethyl, aryloxy, methoxy, CH₂OH, methyl, or C(O)₂H. Preferably, the substituents are halogen, or trifluoromethyl. The substituent halogen groups are preferably Cl, Br and fluorine. More preferably the substituents are in the 3,5- position or the 4-position of the aryl ring. Suitably the

5 substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

When R₇ is an aryl moiety it is preferably phenyl; when R₇ is an arylalkyl moiety it is preferably benzyl; when R₇ is an alkyl moiety it is preferably a methyl or a C₈ unbranched chain. The methyl moiety if substituted is preferably substituted by fluorine to yield a trifluoromethyl group. More preferably R₇ is an optionally substituted phenyl.

1 0 Suitably the aryl substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

When R₇ is NR₉R₁₀ the R₉ group is preferably hydrogen. When R₁₀ is an aryl moiety it is preferably phenyl; when R₁₀ is an aryl alkyl moiety it is preferably benzyl; when R₁₀ is an alkyl moiety it is preferably a methyl or a C₈ unbranched chain. The methyl

1 5 moiety, if substituted, is preferably substituted by fluorine to yield a trifluoromethyl group. More preferably R₁₀ is an optionally substituted phenyl as defined above.

Suitably X is oxygen or sulfur, preferably oxygen.

Suitably R₄ is hydrogen, or C₁₋₄ alkyl, preferably hydrogen.

Suitably R₅ is hydrogen, halogen, CF₃, (CH₂)_tC(O)₂R₈, or (CH₂)_tOH, and t is is

2 0 0 or an integer having a value of 1 or 2. Preferably t is 1. Preferably when R₅ is (CH₂)_tC(O)₂R₈, R₈ is a C₁₋₄ alkyl group, preferably t-butyl. Preferred R₅ groups are hydrogen, CF₃, or halogen. More preferably R₅ is hydrogen or CF₃.

Suitably R₆ is hydrogen or halogen; preferably hydrogen. If R₆ is halogen it is preferably fluorine or chlorine.

2 5

Specifically exemplified compounds of Formula (I) are:

2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5- (1,1-dimethylpropyl)benzenesulfonic acid;

2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-[3-[3,5-

3 0 Bis(trifluoromethyl)phenyl]ureido]phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;

2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;

2-[2-[3-(4-Methoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;

3 5 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid;

2-[2-[3-[3,5-bis(Trifluoromethyl)phenyl]ureido-4-methylphenoxy]-5-(1,1-

- dimethylpropyl)benzenesulfonic acid;
 2-[2-[3-[3,5-bis(trifluoromethyl)phenyl]thioureido]-4-(trifluoromethyl)phenoxy]-5-(1,1-
 dimethylpropyl)benzenesulfonic acid; and
 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethyl)phenoxy]-
 5 benzenesulfonic acid.

For compounds of Formula (II) the variable R₁, R₂, m, t, R₄, R₅, R₆, and R₈ are as preferably as indicated above for compounds of Formula (I).

- Suitably, for compounds of Formula (II), R₃ is -C(O)R₇ or -C(S)R₇ and R₇ is an
 1 0 optionally substituted aryl, an optionally substituted arylC₁₋₂ alkyl, or an optionally substituted C₁₋₈ alkyl group. Preferably R₃ is -C(O)R₇.

- Suitably the aryl, arylalkyl or alkyl moieties of R₇ are substituted independently, one to three times, by halogen, trifluoromethyl, aryloxy, methoxy, CH₂OH, methyl, or C(O)₂H. Preferably, the substituents are halogen, or trifluoromethyl. The substituent halogen groups
 1 5 are preferably Cl, Br and fluorine. More preferably the substituents are in the 3,5- position or the 4-position of the aryl ring. Suitably the substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

- When R₇ is an aryl moiety it is preferably phenyl; when R₇ is an aryl alkyl moiety it is preferably benzyl; when R₇ is an alkyl moiety it is preferably a methyl or a C₈
 2 0 unbranched chain. The methyl moiety if substituted is preferably substituted by fluorine to yield a trifluoromethyl group. More preferably R₇ is an optionally substituted phenyl or benzyl group.

Specifically exemplified compounds of Formula (II) include:

- 2 5 2-[2-(Phenylmethylacetamido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, and its sodium salt
 2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt
 3 0 2-[2-[3,5-bis(trifluoromethyl)benzamido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, and its sodium salt

- Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonic acid, ethane
 3 5 sulphonic acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid. In addition, pharmaceutically acceptable salts of compounds of formula (I)

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may also be formed with a pharmaceutically acceptable cation, if possible. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations.

For purposes herein, the compounds of the generic formulas (I) and (II), for the
5 (R₂)_m term provisos, etc. are numbered by the point of attachment to the ether or thioether. This point of attachment is the one position, the R₁ term is the 2-position, etc. The nomenclature used for naming the specifically exemplified compounds, such as common or IUPAC corresponds to actual rules of nomenclature as is unaffected by the generic formula herein.

10 The following terms, as used herein, refer to:

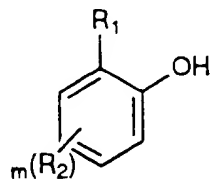
- "halo" - all halogens, that is chloro, fluoro, bromo and iodo;
- "C₁₋₈ alkyl" or "alkyl" - both straight and branched chain radicals of 1 to 8 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like;
- 15 • "aryl" - phenyl and naphthyl;
- "aryl alkyl" or "heteroaryl alkyl" or "heterocyclic alkyl" - is used herein to mean a aryl, heteroaryl or heterocyclic ring connected to a C₁₋₄ alkyl as defined above unless otherwise indicated;
- "heteroaryl" (on its own or in any combination, such as "heteroaryloxy") - a 5-10
20 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, quinoline, isoquinoline, pyridine, pyrimidine, oxazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole;
- "heterocyclic" (on its own or in any combination, such as "heterocyclalkyl") - a
25 saturated or wholly or partially unsaturated 4-10 membered ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O, or S; such as, but not limited to, pyrrolidine, piperidine, piperazine, morpholine, imidazolidine or pyrazolidine. Such rings as noted herein are included in the R₉ and R₁₀ optionally cyclized term, as the ring is limited to only 5 to 7 members.

30

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention.

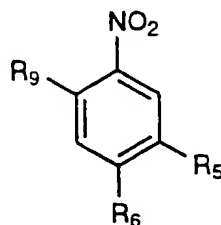
35

Compounds of Formula (I) and (II) may be prepared as described herein by processes which comprises reacting a suitably protected compound of Formula (2), which are generally commercially available and R₁, R₂, m are as defined in Formula (I):



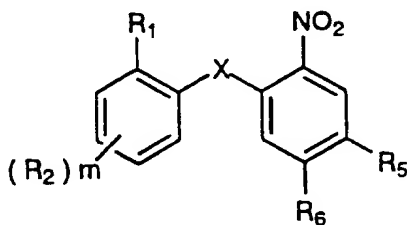
(2)

with a compound of Formula (3) wherein R_9 is F, Cl, Br or I, and R_5 and R_6 are as in Formula (I):



(3)

in a suitable solvent such as dimethylformamide and in the presence of a suitable base such as potassium carbonate with or without added copper at a temperature of 25-175°C to provide a compound of Formula (4). In cases where compounds of Formula (2) are not commercially available, such as in some cases when R_1 is $S(O)_3H$, compounds of formula (2) may be prepared by sulfonating an appropriately substituted phenol with a suitable sulfonating reagent, such as fuming sulfuric acid.

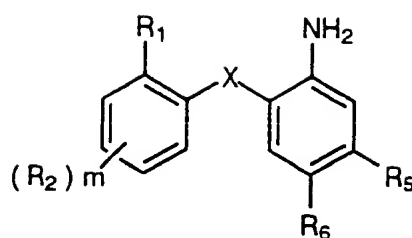


(4)

Alternatively, compounds of Formula (4) where R_1 is $-S(O)_3H$ may be prepared by sulfonation of a compound of Formula (4) where R_1 is H with a suitable reagent such as fuming sulfuric acid. Further, compounds of Formula (I) wherein R_1 is $-SOCH_3$ or $-SO_2CH_3$ may be prepared from compounds of Formula (I) wherein R_1 is $-SCH_3$ by reaction with a suitable oxidizing reagent such as m-chloroperbenzoic acid in a suitable solvent such as methylene chloride.

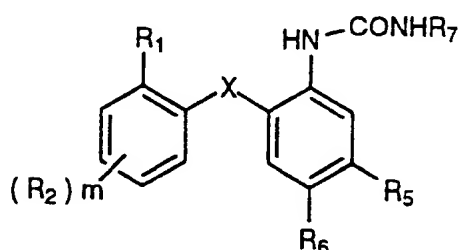
Reduction of compound (4) with a suitable reducing agent such as iron in a solvent such as acetic acid/ethanol/water, or titanium trichloride in acetic acid water, or H_2 in the presence of a catalyst such as palladium on carbon in a solvent such as ethyl acetate or methanol, provides a compound of Formula (5).

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(5)

Reaction of compound (5) with an isocyanate in a suitable solvent such as pyridine provides a compound of Formula (6) wherein R₄ is hydrogen, and R₇ is as defined in Formula (I).

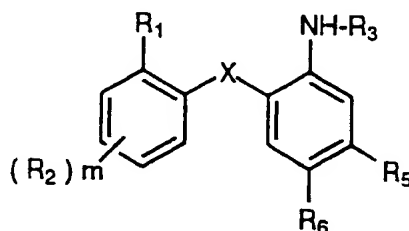


(6)

10

Reaction of a compound of Formula (5) with an alkylating agent or carboxylic acid halide in a suitable solvent such as methylene chloride or pyridine, in the presence of a base, such as triethylamine or pyridine, provides a compound of Formula (8) wherein R₃ is C(O)R₇ and R₇ is an optionally substituted alkyl, aryl or arylalkyl.

15



(8)

Alternatively, compounds of Formula (8), wherein R₃ is C(O)R₇ may be prepared by reacting a compound of Formula (5) with an appropriate aldehyde under dehydrating conditions to form an imine which may be reduced with a suitable reducing agent such as sodium borohydride.

Deprotection (if required) of a compound of Formulas (6-8) and/or conversion to suitable salt forms provides a final compound of Formula (I).

Without further elaboration, it is believed that one skilled in the art can, using procedures analagous to those described herein, utilize the present invention to its fullest extent. The invention will now be described by reference to the following examples which
5 are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

SYNTHETIC CHEMISTRY

Temperatures are recorded in degrees centigrade unless otherwise noted.

10

Example 1

Preparation of 2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

15 a) 2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzene

A mixture of 4-(1,1-dimethylpropyl)phenol (820 mg, 0.005 mol), 4-bromo-3-nitrobenzotrifluoride (1.35 g, 0.005 mol) and potassium carbonate (1.38 g, 0.01 mol), in dimethylformamide was stirred in an oil bath, at 150°C for sixteen hours. The solvent was evaporated and the residue partitioned between ethyl acetate and water. The phases were
20 separated and the aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were dried over MgSO₄ and evaporated. The residue was flash chromatographed (silica gel, ethyl acetate/hexane) to give the title compound. ¹H NMR (250 MHz, CDCl₃) δ 8.22 (d, 1H), 7.68 (dd, 1H), 7.48 (dd, 2H), 7.03 (d, 3H), 1.62 (q, 2H), 1.3(s, 6H), 0.70 (t, 3H).

25

b) 2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

Sulfuric acid (15% SO₃) (10 ml) was added slowly to 2-(2-nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzene (7.67 g, 0.02 mol) over
30 approximately an hour, at 0°C. After the addition was complete the mixture was allowed to stir for an hour, at 0°C. The reaction mixture was then quenched in ice water and the aqueous mixture was extracted (3x) with ethyl acetate. The organic phase was dried over MgSO₄, filtered and evaporated. The residue was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound - a yellow
35 solid. ¹H NMR (250 MHz, CDCl₃) δ 7.95 (d, 1H), 7.90 (d, 1H), 7.65 (dd, 1H), 7.39 (dd, 1H), 7.0 (d, 1H), 6.82 (d, 1H), 1.65 (q, 2H), 1.25 (m, 6H), 0.7 (t, 3H).

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c) 2-(2-Amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid

A mixture of 2-(2-nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)-benzenesulfonic acid, ammonium salt (6.82 g 0.015 mol) and 10% palladium on carbon (2.22 g) in ethyl acetate (400 ml) was hydrogenated in a parr bottle at 55 psi, for two hours.

5 The mixture was diluted with chloroform under argon, filtered through Celite® and the filtrate evaporated to give the title compound as a white solid. ¹H NMR (250 MHz, CD₃OD) δ 7.95 (d, 1H), 7.34 (dd, 1H), 7.09 (m, 1H), 6.85 (m, 1H), 6.75 (d, 2H), 1.65 (q, 2H), 1.25 (m, 6H), 0.65 (t, 3H).

10 d) 2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid, sodium

2-(2-Amino-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid (340 mg, 0.0008 mol) and phenyl isocyanate (110 µl, 0.0009 mol) were mixed in pyridine (5 ml) at room temperature for sixteen hours. The solvent was evaporated and the residue
15 was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The residue was dissolved in water/methanol and sodium bicarbonate was added. The liquid was evaporated and this was repeated one more time. The residue was flash chromatographed (C₁₈ reverse phase, MeOH/H₂O) to give the title compound. MS (FAB) m/e 545 [M+H]⁺, m/e 1045 [2M+H]⁺

20

Example 2

Preparation of 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

25

a) 2-Hydroxy-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

Fuming sulfuric acid (10 mL) and methylene chloride (10 mL) were combined and cooled with stirring to -20°C; and 4-(1,1-dimethylpropyl)phenol (10 g, 6 mmol) was added. After stirring for 15 min, the reaction mixture was quenched on ice and the crude product
30 was purified by flash chromatography (silica gel, methylene chloride/ethanol/ammonium hydroxide) to give the title compound. ¹H NMR (250 MHz, CD₃OD/CDCl₃) δ 7.65 (d, 1H), 7.26 (dd, 1H), 6.82 (d, 1H), 1.65 (q, 2H), 1.28 (s, 6H), 0.67 (t, 3H).

b) 2-(2-Nitrophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

35 A mixture of 2-hydroxy-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (1.1 g, 5.76 mmol), 1-fluoro-2-nitrobenzene (1.2 mL, 8 mmol), and potassium carbonate (1.52 g, 11 mmol) in dimethylformamide (10 mL) was stirred under argon and

heated in an oil bath to 150C° for 90 min. The reaction mixture was diluted with ethyl acetate, filtered, and the residue washed with ethyl acetate. The combined filtrates were evaporated and the crude product was purified by flash chromatography (silica gel, CH₂Cl₂/isopropanol/NH₄OH) to give the title compound. ¹H NMR (250 MHz, CD₃OD/CDCl₃) 8.00 (d, 1H), 7.93 (dd, 1H), 7.42-7.55 (m, 2H), 7.20-7.28 (m, 1H), 7.04 (dd, 1H), 6.92 (d, 1H), 1.70 (q, 2H), 1.32 (s, 6H), 0.72 (t, 3H).

c) 2-(2-Aminophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid

A mixture of 2-(2-nitrophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (26 g, 0.076 mol) and 10% palladium on carbon (1 g) in ethyl acetate (400 mL) was hydrogenated in a parr bottle at 55 psi for 2 h. The reaction mixture was flushed with argon, filtered through Celite® and evaporated. The crude product was purified by flash chromatography (silica gel, ethyl acetate/hexane) to give the title compound as a white solid.

¹H NMR (250 MHz, CD₃OD/CDCl₃) δ 7.93 (d, 1H), 7.27 (dd, 1H), 6.66-7.06 (m, 5H), 1.65 (q, 2H), 1.27 (s, 6H), 0.68 (t, 3H).

d) 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

The 2-(2-aminophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid (0.5 g, 1.5 mmol) was dissolved in pyridine (40 mL) and was stirred under argon at room temperature. 3,5-Bis(trifluoromethyl)benzenesulfonyl chloride (0.6 g, 2 mmol) was added and the mixture stirred for 16 h. The solvent was evaporated, and the residue was purified by flash chromatography (silica gel, methylene chloride/ethanol/ammonium hydroxide) to give the title compound. ¹H NMR (250 MHz, CD₃OD/CDCl₃) δ 8.18 (dd, 1H), 8.04 (s, 2H), 7.98 (d, 1H), 7.46 (s, 1H), 7.06-7.34 (m, 4H), 6.83 (d, 1H), 1.63 (q, 2H), 1.27 (s, 6H), 0.61 (t, 3H).

e) 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

A solution of 2-[2-(3-phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (0.39 g, 0.62 mmol) in 1:1 methanol/water (10 mL) was stirred under argon. Sodium carbonate (84 mg, 1 mmol) was added and the mixture stirred for 15 min. The solvents were evaporated and the residue was flash chromatographed (C₁₈ reverse phase, MeOH/H₂O) to give the title compound after lyophilization. MS (FAB) m/e 477.2 [M+H]⁺

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Example 3Preparation of 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

- 5 a) 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

2-(2-Amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid (22 mg, 0.0005 mol) was dissolved in pyridine (5 ml) and stirred under argon. 4-Phenoxyphenylisocyanate (103 μ l, 0.0006 mol) was added and the reaction was stirred for
1 0 sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The fractions containing product were evaporated and the residue mixed with water/methanol and sodium bicarbonate was added. The liquid was evaporated, and this was repeated one time. The residue was flash chromatographed (C 18 reverse phase, MeOH/H₂O) to give the title compound. MS
1 5 (FAB) m/e 637 [M+H]⁺, m/e 659 [M+Na]⁺.

Example 4

2 0 Preparation of 2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

- a) 2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

Following the procedure of Example 3 except substituting benzoyl chloride for 4-phenoxyphenylisocyanate, the title compound was obtained. MS (FAB) m/e 530 [M+H]⁺,
2 5 m/e 552 [M+Na]⁺.

Example 5

3 0 Preparation of 2-[2-[3,5-Bis (trifluoromethyl)benzamido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

- a) 2-[2-[3,5-Bis(trifluoromethyl)benzoamido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

3 5 Following the procedure of Example 3 (a) except substituting 3,5-bis(trifluoromethyl)benzoyl chloride for 4-phenoxyphenyl isocyanate, the title compound was obtained. MS (FAB) m/e 688 [M+Na]⁺, m/e 1353 [2M+Na]⁺.

Example 6

Preparation of 2-[2-[3-[3,5-Bis (trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, sodium salt

5

a) 2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1,3,3-tetramethylbutyl)benzene

A mixture of 4-(*tert*-octyl)phenol (500 mg, 0.0024 mol), 4-bromo-3-nitrobenzotrifluoride (648 mg, 0.0024 mol) and potassium carbonate (663 mg, 0.0048 mol) in dimethylformamide (25 ml) was stirred in an oil bath, at 150°C for sixteen hours. The cooled mixture was filtered and the filtrate evaporated. The residue was flash chromatographed (silica gel, ethyl acetate/hexane) to give the title compound. ¹H NMR (250 MHz, CD₃OD) δ 8.25 (d, 1H), 7.8 (dd, 1H), 7.5 (tt, 2H), 7.05 (m, 3H), 1.85 (s, 2H), 1.4 (s, 6H), 0.75 (s, 9H).

1 5 b) 2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt

2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1,3,3-tetramethylbutyl)benzene (590 mg, 0.0015 mol) was dissolved in methylene chloride and slowly dripped into a 1:1 mixture of sulfuric acid/methylene chloride at 0°C. After the addition was complete the mixture was stirred for ten minutes at 0°C, noting a color change - yellow to purple. The reaction mixture was quenched in ice and the aqueous mixture extracted with ethyl acetate (3x). The organic phase was dried over MgSO₄, filtered, and evaporated. The crude product was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound. ¹H NMR (250 MHz, CD₃OD) δ 8.2 (d, 1H), 8.07 (d, 1H), 7.72 (dd, 1H), 7.52 (dd, 1H), 7.0 (m, 2H), 1.82 (s, 2H), 1.4 (s, 6H), 0.8 (s, 9H).

2 5 c) 2-(2-Amino-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt

2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt (650 mg, 0.0013 mol) was dissolved in ethyl acetate (30 ml) and 10% palladium on carbon (125 mg) was added. The mixture was hydrogenated at 55 psi, in a parr bottle, for three hours. The reaction mixture was diluted with chloroform under argon, filtered through Celite® and the filtrate evaporated. The residue was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound. ¹H NMR (250 MHz, CD₃OD) δ 8.0 (d, 1H), 7.35 (dd, 1H), 7.05 (m, 2H), 6.9 (dd, 1H), 6.75 (d, 1H), 1.75 (s, 2H), 1.40 (s, 6H), 0.75 (s, 9H).

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d) 2-[2-[3-[3,5-Bis(trifluoromethyl)phenoxy]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, sodium salt

A mixture of 2-(2-amino-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt (440 mg, 0.001 mol), 3,5-bis(trifluoromethyl)phenyl isocyanate (364 μ l, 0.0022 mol) and pyridine (12 ml) was stirred at room temperature for sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The residue was dissolved in water/methanol and sodium bicarbonate was added and the liquid evaporated. This was repeated to give title compound. MS (ES) m/e 701 [M+H]⁺, m/e 723 [M+Na]⁺.

Example 7

Preparation of 2-[2-[3-[3,5-Bis (trifluoromethyl)phenyl]ureido]-4-methylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

a) 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-methylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

Following the procedure of Example 3 except substituting 3,5-bis(trifluoromethyl)phenyl isocyanate for 4-phenoxyphenyl isocyanate, the title compound was obtained. MS (ES) m/e [M+2H-Na]⁺, m/e 627 [M+H]⁺

Example 8

Preparation of 2-[2-[3-[3,5-Bis (trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]benzenesulfonic acid, sodium salt

a) 2-(2-Nitro-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt

A mixture of 2-hydroxybenzenesulfonic acid, ammonium salt (500 mg, 0.0026 mol), 4-fluoro-3-nitrobenzotrifluoride (746 μ l, 0.0053 mol) and potassium carbonate (719 mg, 0.005 mol) in dimethylformamide (20 ml) was stirred at 150°C, in an oil bath, for sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound. ¹H NMR (250 MHz, CDCl₃) δ 8.25 (s, 1H), 8.05 (d, 1H), 7.7 (dd, 1H), 7.5 (m, 1H), 7.35 (t, 1H), 7.08 (m, 2H).

b) 2-(2-Amino-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt

2-(2-Nitro-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt (670 mg) was dissolved in ethyl acetate (20 ml) and 10% palladium on carbon (335 mg) was added under argon. The mixture was hydrogenated, in a parr bottle, at 55 psi for three hours. The reaction mixture was diluted with chloroform under argon, filtered through Celite® and the filtrate evaporated. The residue was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound. ¹H NMR (250 MHz, CDCl₃) δ 8.0 (dd, 1H), 7.35 (t, 2H), 7.1 (m, 2H), 6.95 (dd, 1H), 6.82 (d, 1H).

1 0 c) 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-(trifluoromethyl)phenoxy]-benzenesulfonic acid, sodium salt

A mixture of 2-(2-amino-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt (400 mg, 0.0011 mol) and 3,5-di(trifluoromethyl)phenyl isocyanate in pyridine (10 mL) was stirred at room temperature for sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The fractions containing product were evaporated and the residue dissolved in water/methanol and sodium bicarbonate added. The liquid was evaporated and this was repeated one time to give the title compound. ¹H NMR (500 MHz, CD₃OD) δ 8.85 (d, 1H), 8.1 (s, 2H), 7.95 (dd, 1H), 7.55 (s, 1H), 7.40 (dd, 1H), 7.35 (tt, 1H), 7.28 (d, 1H), 7.15 (dt, 1H), 6.95 (dd, 1H).

Example 9

Preparation of 2-[2-[3,5-Bis(trifluoromethyl)phenylmethylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

2 5

a) 3,5-bis(trifluoromethyl)phenyl acetyl chloride

3,5-bis(trifluoromethyl)phenylacetic acid (1.09 g, 4 mmol) was dissolved in methylene chloride (10 mL) at -10°C and stirred under argon. Oxalyl chloride (0.436 mL, 5 mmol) and dimethylformamide (0.01 mL) were added and the mixture stirred until gas evolution ceased. The solvents were evaporated and the crude product was used in the next step without further purification.

3 0

b) 2-[2-[3,5-Bis(trifluoromethyl)phenylmethylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

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2-(2-amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (0.3 g, 0.74 mmol) was dissolved in methylene chloride (4 mL) and stirred under argon at -40°C. Pyridine (0.061 mL, 0.75 mmol) was added followed by the

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dropwise addition of 3,5-bis(trifluoromethyl)phenyl acetyl chloride (232 mg, 0.8 mmol) in methylene chloride (1 mL). The solvent was evaporated and the crude product was purified by flash chromatography (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title product. ¹H NMR (250 MHz, CDCl₃) δ 8.62-8.64 (d, 1H), 8.00-8.02 (d, 1H), 7.96 (s, 2H), 7.81 (s, 1H), 7.31-7.46 (m, 3H), 6.88-6.92 (d, 1H).

c) 2-[2-[3,5-Bis(trifluoromethyl)phenylmethylacetamido-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

A solution of 2-[2-[3,5-bis(trifluoromethyl)phenylmethylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (0.28 g, 0.41 mmol) in 1:1 methanol/water (10 mL) was stirred under argon. Sodium carbonate (55 mg, 0.65 mmol) was added and the mixture stirred for 15 min. The solvents were evaporated and the residue was flash chromatographed (C₁₈ reverse phase, MeOH/H₂O) to give the title compound after lyophilization. MS (FAB) m/e 680.4 [M+H]⁺.

The following compounds may also be made by one of skill in the art using methods analogous to the Examples indicated above.

Example 10 : 2-[2-(Phenylmethylacetamido)phenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid

Example 11 : 2-[2-[3-[3,5-bis(trifluoromethyl)phenyl]thioureido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid

Example 12: 2-[2-[3-(4-Methoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid

Example 13: 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]phenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid, and its sodium salt.

Example 14: 2-[2-(Nonanamido)phenoxy]benzoic acid; m.p. 81-82 °C

Example 15: 2-[2-[3,5-Bis-(trifluoromethyl)]benzamido-4-trifluoromethylphenoxy]-benzoic acid; m.p. 178 °C

METHODS OF TREATMENT

The compounds of Formula (I)/(II), or pharmaceutically acceptable salts thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of an inflammatory disease state in a mammal, preferably a human.

Inhibition of PLA₂ and/or CoA-IT and the simultaneous reduction of PAF, free arachidonic acid and eicosanoid release from inflammatory cells according to this invention is of therapeutic benefit in a broad range of diseases or disorders. The invention herein is

therefore useful to treat such disease states both in humans and in other mammals.

Inhibition of CoA-IT and PLA₂ by the compounds of Formula (I)/(II) is an effective means for simultaneously reducing PAF, free arachidonic acid and eicosanoids produced in inflammatory cells. The therapeutic utility of blocking lipid mediator generation has been recognized for many years. For example, inhibitors of cyclooxygenase, such as aspirin, indomethacin, acetaminophen and ibuprofen, have demonstrated broad therapeutic utilities. CoA-IT inhibitors inhibit cyclooxygenase products. Another class of inhibitors which are used in a broad range of inflammatory disorders are the corticosteroids. Corticosteroids act in a variety of ways, e.g. to induce inflammatory cells to produce proteins which inhibit free arachidonic acid release or to down regulate PLA₂ mRNA formation. CoA-IT inhibitors block the release of free arachidonic acid. Inhibitors of 5-lipoxygenase block the production of leukotrienes and leukotriene antagonists prevent the bioactions of leukotrienes. Recent studies indicate that both will have broad therapeutic utilities, and CoA-IT inhibitors block the production of leukotrienes. Inhibitors of phospholipase A₂ block the release of free arachidonic acid and the formation of lyso PAF (the immediate precursor of PAF). PLA₂ inhibitors are recognized to have broad therapeutic utilities. It does not, however, follow that the disease states noted above are in fact caused by altered CoA-IT activity or PLA₂. Thus, the disease state itself may not be directly mediated by CoA-IT or PLA₂ activity. It only follows that CoA-IT or PLA₂ activity is required for the continued expression of symptoms of the disease state and that CoA-IT or PLA₂ inhibitors will be beneficial against the symptoms of these disease states.

Recognition that CoA-IT reduces PAF production has a number of therapeutic implications. PAF itself has been implicated as being involved in a number of medical conditions. Thus in circulatory shock, which is characterised by systemic hypotension, pulmonary hypertension and increased lung vascular permeability, the symptoms can be mimicked by infusion of PAF. This coupled with evidence showing that circulating PAF levels are increased by endotoxin infusion indicate that PAF is a prime mediator in certain forms of shock.

Intravenous infusion of PAF at doses of 20-200 pmol kg⁻¹ min⁻¹ into rats has been reported to result in the formation of extensive haemorrhagic erosions in the gastric mucosa. Thus PAF is the most potent gastric ulcerogen yet described whose endogenous release may underlie or contribute to certain forms of gastric ulceration. Psoriasis is an inflammatory and proliferative disease characterised by skin lesions. PAF is pro-inflammatory and has been isolated from lesioned scale of psoriatic patients indicating PAF has a role in the disease of psoriasis. And finally, increasing evidence supports a potential patho-physiological role for PAF in cardiovascular disease. Thus recent studies in angina patients show PAF is released during atrial pacing. Intracoronary injection of PAF in pigs

induces a prolonged decrease in coronary flow and, in guinea pig hearts, it induces regional shunting and ischaemia. In addition PAF has been shown to initiate thrombus formation in a mesenteric artery preparation, both when administered exogenously and when released endogenously. More recently PAF has been shown to play a role in brain ischaemia induced in animal models of stroke.

Thus the compounds of the invention, by virtue of their ability to antagonise either CoA-IT and/or PLA₂, thus block the production of PAF, free arachidonic acid and its metabolites, are likely to be of value in the treatment of any of the above conditions.

Disease states which could benefit from the inhibition of lipid mediator production include, but are not limited to, adult respiratory distress syndrome, asthma, arthritis, reperfusion injury, endotoxic shock, inflammatory bowel disease, allergic rhinitis and various inflammatory skin disorders. Each of these disorders is mediated in some part by lipid mediators of inflammation. Compounds which inhibit CoA-IT, by virtue of their ability to block the generation of lipid mediators of inflammation, are of value in the treatment of any of these conditions. Similarly compounds which inhibit PLA₂, by virtue of their ability to block the generation of lipid mediators of inflammation stemming from release of this enzyme are of value in the treatment of these conditions. In particular, an inhibitor of PLA₂, for instance would offer an advantage over the classical NSAIDs which affect only prostanoid production (and not PAF biosynthesis) thereby inhibiting both the acute and cell-mediated "chronic" inflammatory processes. Likewise, selective inhibition of COX-2, by compounds herein, is useful for the treatment of diseases mediated thereby, such as arthritis, and IBD, for relief of pain, and inflammation, without the gastric and renal side effects associated with COX-1 inhibition.

Treatment of disease states caused by these lipid inflammatory mediators i.e., arachidonate, eicosanoids and PAF, include certain cardiovascular disorders such as but not limited to, myocardial infarction, stroke, circulatory shock, or hypotension, ischemia, reperfusion injury, inflammatory diseases such as, but not limited to, arthritis, inflammatory bowel disease, Crohn's disease, or ulcerative colitis, respiratory disease such as but not limited to, asthma, or adult respiratory distress syndrome, anaphylaxis, shock such as but not limited to endotoxic shock, topical diseases, such as but not limited to actinic keratosis, psoriasis, or contact dermatitis, or pyresis.

In order to use a compound of formula (I) or (II) a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of formula (I) and a pharmaceutically acceptable carrier or diluent.

Compounds of formula (I)/(II), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of formula (I)/(II) may be administered in conventional dosage forms prepared by combining a compound of formula (I)/(II), with standard pharmaceutical carriers according to conventional procedures. Such pharmaceutically acceptable carriers or diluents and methods of making are well known to those of skill in the art, and reference can be found in such texts as Remington's Pharmaceutical Sciences, 18th Ed., Alfonso R. Genarao, Ed., 1990, Mack Publishing Co. and the Handbook of Pharmaceutical Excipients, APhA Publications, 1986.

The compounds of formula (I)/(II) may also be administered in conventional dosages in combination with known second therapeutically active compounds, such as steroids or NSAID's for instance. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25mg. to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of formula (I)/(II) may be administered topically, that is by non-systemic administration. This includes the application of a compound of Formula (I)/(II), externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from
5 0.001% to 10% w/w, for instance from 1% to 2% by weight of the formulation. It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the formulation.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing
1 0 a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid
1 5 formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as
2 0 almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as
2 5 siliceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may
3 0 then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C. for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and
3 5 chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Each dosage unit for oral administration contains preferably from 1 to 250 mg (and

for parenteral administration contains preferably from 0.1 to 25 mg) of a compound of the Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For an adult patient this may be, for example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the Formula (I)/(II), or a pharmaceutically acceptable salt thereof calculated as the free base, the compound being administered from 1 to 4 times per day.

It will be recognized by one of skill in the art that the optimal quantity the individual dosages of the compounds used herein will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques by those skilled in art. The dosage to be administered is obviously dependent upon species, body weight, age, individual condition and method of application. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses given per treatment, the frequency of such treatment and the eventual duration of use can be ascertained by those skilled in the art using conventional course of treatment determination tests.

BIOLOGICAL METHODS:

To determine activity of the compounds of Formula (I) and (II) various cellular assays can be used to determine in vitro activity. Additionally, various classical in vivo acute inflammatory models which have some aspect of their etiology to elevated eicosanoid levels can be employed, such as the paw edema model, mouse zymosan peritonitis, reverse Arthus pleurisy or various skin inflammation assays which are described in Lewis et al., Experimental Models of Inflammation, in the Handbook of Inflammation, Vol. 5, Bonta Ed., Elsevier Science Publishers, NY (1985) whose disclosure is herein incorporated by reference. The TPA induced ear edema model (mouse) as well as the carrageenan paw edema model in the rat are described herein as well. These classical models of inflammation will reflect the drug's ability to alter an inflammatory response but cannot address the specificity of drug action. These models have been traditionally designed as non steriod antiinflammatory drug sensitive pharmacological screens and it is important to utilize models which can differentiate PLA2 and CoA-IT inhibitors from NSAIDS.

Cell-free and Cellular Assessment of Inhibitors

Described herein are several in vitro assays both for CoA-IT and PLA2 enzyme activities.

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The first employees purified recombinant enzyme or a broken cell assay, assay (a or b, respectively) described below. Alternatively, evaluation of inhibitors can occur in intact cells such as described in the assay, assay (c and d) below. CoA-IT activity can exclusively be measured, and differentiated from PLA₂ inhibition, in intact cells by following the movement of a pulse of [³H] arachidonate as it moves into the 1-alkyl and 1-alkenyl phospholipids in inflammatory cells (assay e). It should be noted for the purposes herein that assays c, d, & f can both be used for PLA₂ and CoA-IT inhibition determination.

Inflammatory Responses *in vivo*

The ability of compounds that inhibit CoA-IT and/or PLA₂ to affect *in vivo* inflammatory responses may be assessed. Inflammatory responses are induced in the mouse ear by the topical application of a pro-inflammatory agent, such as 12-O-tetradecanoylphorbol 13-acetate (assay g). This produces an edematous response, as measured by increases in ear thickness, as well as increased inflammatory cellular infiltrate, as measured by increases in myeloperoxidase activity (as described in the methods). To further validate the mechanism of action inflammation induced by the direct administration of arachidonic acid can be used. In this case, compounds altering arachidonic acid mobilization or liberation should be without effect.

In Vitro Assays

Assay (a): Phospholipase A₂ assay:

Phospholipase A₂ activity of rh Type II - 14 KDaPLA₂ or PLA₂ semi-purified from human Synovial joint fluid was measured by the acylhydrolysis of high specific activity (NEN)[³H]-AA-*E. coli* (0.5 mCi/5nmol PL Pi) as previously described in Marshall et al., J. Rheumatology, 18:1, pp59-65 (1991). High specific activity [³H]AA-*E. coli* had up to 95% of the label incorporated into phospholipid which was localized almost exclusively in the sn-2 position, as demonstrated by purified 14kDa PLA₂ or low molecular weight PLA₂ acylhydrolysis and separation of products by thin layer chromatography (TLC) (data not shown). [Predominately used herein was rh Type II 14 kDa PLA₂, or alternatively bovine pancreatic PLA₂ was also be used]. The reaction mixture (50 or 100 ml total volume) contained 25 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM CaCl₂ and [³H]-AA-*E. coli* (low specific activity; 5-8 nmol PL Pi per assay). Assays were incubated for a time predetermined to be on the linear portion of a time versus hydrolysis plot (10 min). Experiments were conducted with final % hydrolysis values ranging from 2% (400-1000 dpm) to 10% (2000-5000 dpm) acylhydrolysis after blank correction. Reactions were terminated by the addition of 1.0 mL tetrahydrofuran (THF). The whole sample was placed

over aminopropyl solid phase silica columns and eluted with THF:acetic acid (49:1) exclusively separating free fatty acids with greater than 95% recovery. Radiolabel in this eluate was quantitated by liquid scintillation counting. Results were expressed as % of fatty acid hydrolyzed ($[(\text{sample dpms} - \text{non-specific (blank) dpms}) / \text{total dpms}] \times 100$) or specific activity which was calculated from hydrolysis values found in the linear portion of time versus % hydrolysis plots (pmol free fatty acid hydrolyzed/mg/min). Non-specific activity was always less than 1% of the total counts added.

Protein determination

1 0 All protein concentrations were determined by Bradford protein analysis kits (Biorad, Richmond, CA).

Results:

1 5 The following representative compounds of Formula (I) and (II) below demonstrated positive PLA₂ inhibition in the above noted method. While these compounds generally tested positive at 50 μM levels, several were also tested for positive inhibitory activity at up to 500 μM levels. Such compounds include all compounds of Examples 1 to 13 herein.

2 0 Assay (b) : CoA-IT Activity

The following is a method to measure CoA-IT activity and the effects of compounds on CoA-IT activity. The assay is based upon mixing cellular material containing CoA-IT activity with a stable lyso phospholipid such as 1-alkyl-2-acyl-GPC and measuring the production of phospholipid product such as 1-alkyl-2-acyl-GPC occurring in the absence of added CoA or CoA-fatty acids.

Cell Preparation

Any inflammatory cell that contains high levels of CoA-IT activity can be used, such as neutrophils, macrophages or cell lines such as U937 cells. U937 cells were obtained from American Type Culture Collection and grown in RPMI-1640 media (Gibco, Grand Island, New York) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37°C, 5%CO₂. Cells were grown without differentiation (basal state) by any agent, such as dimethyl sulfoxide. As used herein, "inflammatory cells" include, but are not limited to neutrophils, macrophages, monocytes, lymphocytes, eosinophils, basophils, and mast cells.

Microsomal preparation

Microsomes were prepared using standard techniques. In this case, cells were washed with a buffer of 250 mM sucrose, 10 mM Tris, 1 mM EGTA, 1 mM MgCl₂, pH 7.4 and ruptured by N₂ cavitation (750 psi, 10 minutes). The ruptured cells were centrifuged 1000 X g, 5 minutes. The resulting supernatant was centrifuged at 20,000 X g, ~20 minutes. Microsomes were prepared from this supernatant by centrifugation at 100,000 x g, 60 minutes. The resulting pellet was washed once with assay buffer (150 mM NaCl, 10 mM Na₂KPO₄, 1 mM EGTA, pH 7.4), recentrifuged and the pellet resuspended in assay buffer (4-20 mg protein/ml) and was stored at -80°C until assayed.

10 CoA-IT activity

CoA-IT activity was measured in 1.5 ml centrifuge tubes in a total volume of 100 ul. Microsomes were diluted in assay buffer to the desired protein concentration (6-20 ug/tube). The reaction was initiated by addition of [3H] 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (GPC) (~ 0.1 uCi/tube) and 1 µM final cold 1-alkyl-2-lyso-GPC in assay buffer with 0.25 mg/ml fatty acid-poor bovine serumalbumin (BSA) (Calbiochem, La Jolla, CA). [3H]1-alkyl-2-lyso-GPC, approximately 50 Ci/mmol, was from NEN-Dupont (Boston, Massachusetts) and cold 1-alkyl-2-lyso-GPC was from Biomol (Plymouth Meeting, Pennsylvania). Microsomes were pretreated with desired agents for the desired time (10 minutes) before the addition of [3H]1-alkyl-2-lyso-GPC. The reaction was run for the desired time (10 minutes) at 37°C. The reaction was stopped and the lipids extracted by addition of 100 ul of chloroform:methanol (1:2, v/v) followed by 100 ul of chloroform and 100 ul of 1 M KCl. The samples were vortexed and centrifuged at high speed in a microfuge for 2-3 minutes. An aliquot of the chloroform-extracted materials were separated, usually by TLC in chloroform/methanol/acetic acid/water (50:25:8:4, v/v), visualized by radioscanning (Bioscan) and the product, [3H] 1-alkyl-2-acyl-GPC, was scraped and quantified by liquid scintillation spectroscopy. With this TLC system, the synthetic standards of 1-alkyl-2-lyso-GPC and 1-alkyl-2-acyl-GPC were well separated, with R_f values of approximately 0.25 and 0.65, respectively. Other methods can be used to separate substrate from product, including but not limited to column chromatography, affinity chromatography and post reaction derivitization.

Protein concentration were assessed using the protein assay reagents from Bio-Rad (Richmond, California).

35 Results

A variety of compounds have been tested in this assay to determine its selectivity and inability to detect trivial, non-selective inhibitors. Inhibitors of 5-lipoxygenase (5-LO) and cyclooxygenase (CO), such as indomethacin, naproxen, 6-(4'-Fluorophenyl)-5-(4-pyridyl)-

2,3-dihydroimidzo-[2,1-b]thiazole and 6-(4'-Fluorophenyl)-5-(4-pyridyl)2,3-dihydroimidzo-[2,1-b]thiazole-dioxide had no effect on CoA-IT activity at concentrations up to 100 μ M. The anti-oxidant BHT also has no effect at concentrations up to 100 μ M. Compounds which complex with phospholipids and inhibit PLA₂ activity, such as

5 quinacrine and aristolochic acid have no effect on CoA-IT activity at concentrations up to 500 μ M. Doxepine, a compound reported to inhibit PAF release did not inhibit CoA-IT at concentrations up to 100 μ M. Sodium diclofenac, reported to decrease leukotriene production by altering arachidonic acid metabolism, had no effect on CoA-IT activity at concentrations up to 500 μ M. These results show that the assay for CoA-IT activity is

10 sensitive and selective.

Representative compounds of Formula (I) and (II) Representative compounds of Formula (I) which inhibit CoA-IT activity in the microsomal CoA-IT assay (assay (a) above) [generally at 50 μ M or less] are the compounds:

15 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;

20 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid;
2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-benzenesulfonic acid;
2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;

25 and
2-[2-[3,5-bis(trifluoromethyl)benzamido-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid.

Assay (c): Arachidonic Acid Release Assay

30 Preparation of human neutrophils

Human neutrophils are obtained in the laboratory using three different methods. One method uses leukopheresis packs from normal humans and neutrophils are isolated using the histopaque-1077 technique. The blood is centrifuged at 300 x g for 10 minutes. The cell pellets are resuspended in PBS composed of 137 mM NaCl, 8.8 mM Na₂HPO₄,

35 1.5 mM KH₂PO₄, 2.7 mM KCl (Dulbecco's Gibco Laboratories, Long Island, New York) and layered over histopaque-1077 (Sigma, St. Louis, Missouri). The pellets are collected after centrifugation (300 x g for 30 minutes) and washed once in PBS. The cell pellets are

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exposed briefly to deionized water to lyse any erythrocytes. The remaining cells are collected by centrifugation, suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

- 5 The second method isolates human neutrophils from fresh heparinized normal blood using the Histopaque-1077 technique. The blood is layered over Histopaque-1077 (Sigma, St. Louis Missouri) and centrifuged at 400 x g for 30 minutes. The cell pellets are resuspended in 35 ml of PBS and 12 ml of 6% Dextran, followed by Dextran sedimentation at room temperature for 45 minutes. The upper layer is collected and further centrifuged
- 1 0 for 10 minutes at 1000 rpm. The cell pellets are exposed briefly to deionized water to lyse erythrocytes. The remaining cells are collected by centrifugation, suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

- The third method isolates human neutrophils from freshly drawn heparinized normal
- 1 5 blood using the Percoll technique. The blood is first treated with 6% Dextran at room temperature for a 1 hour sedimentation. The upper layers of plasma are collected and centrifuged at 400 x g for 10 minutes. The cell pellets are resuspended in Percoll 1.070 g/ml supplemented with 5% fetal bovine serum and layered on discontinuous gradients (1.080, 1.085, 1.090, 1.095 g/ml) followed by centrifugation at 400 x g for 45 minutes.
- 2 0 The neutrophils are collected from interfaces of 1.080 and 1.085 and the 1.085 and 1.090 Percoll densities, followed by a centrifugation at 400 x g for 45 minutes. The neutrophils are suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

- There should be no difference noted in the response of the neutrophils nor in the
- 2 5 effects of test compounds in neutrophils isolated by the three different techniques.

Treatment of human neutrophils

- Neutrophils are suspended in PBS with 1 mM Ca^{2+} and 1.1 mM Mg^{2+} at concentrations of 5 to 20 x 10⁶ cells per ml. Cells are added to test tubes and treated with
- 3 0 the desired compounds for 5 to 10 minutes, then challenged with calcium ionophore A23187, 2 μM , or vehicle control, PBS containing 0.25-1 mg/ml BSA. After 5 to 20 minutes, the reactions are terminated by addition of an equal volume of chloroform:methanol (1:2, v/v) to the samples. [²H]Arachidonic acid (50, 100 or 200 ng) is added as an internal standard and the lipids were extracted by addition of equal volumes of chloroform and
- 3 5 distilled water. The samples are vortexed and centrifuged at high speed and the chloroform layer removed to a clean tube.

Assay for free arachidonic acid

The chloroform extract for each sample is evaporated to dryness and the material resuspended in hexane. The hexane is passed through a Silica solid phase column (500 mg), washed 2x with hexane and a fatty acid enriched fraction eluted with hexane:ethyl ether (1:1, v/v). Solvents are removed from the samples under a stream of nitrogen then the samples are converted to pentafluorobenzyl esters using pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile. Solvents are removed and samples are suspended in hexane. GC/MS analysis is performed on a suitable instrument, such as a Finnigan MAT TSQ 700 GC/MS/MS/DS (San Jose, California) operated as a single stage quadrupole system or a Hewlett-Packard 5890 with a 5989A MS system.

The peaks corresponding to arachidonic acid and [$^2\text{H}_8$]Arachidonic acid are identified and the areas of those peaks compared and the released arachidonic acid calculated as ng of arachidonic acid for each sample.

Protein concentrations are assessed using the protein assay reagents from Bio-Rad (Richmond, CA).

Assay (d): Assay for Production of Platelet-Activating Factor (PAF)

Preparation of human neutrophils:

Blood is obtained from normal humans and neutrophils are isolated as described for the arachidonic acid release assay, above. The final leukocyte preparation should be of greater than 95% purity and viability.

Treatment of human neutrophils

Neutrophils are suspended in PBS at concentrations of 5 to 20×10^6 cells per ml. Cells are added to test tubes and treated with the desired compounds for 5 to 10 minutes, then challenged with calcium ionophore A23187, $2 \mu\text{M}$ and 20-30 μCi of [^3H]acetic acid (NEN-Dupont, Boston, Massachusetts), or the vehicle of PBS with 0.25-1 mg/ml. After 5 to 20 minutes, the reactions are terminated by addition of an equal volume of chloroform:methanol (1:2, v/v) to the samples and the lipids are extracted by addition of equal volumes of chloroform and distilled water. The samples are vortexed and centrifuged at high speed and the chloroform layer removed to a clean tube.

Assay for PAF

The chloroform from each tube is evaporated to dryness and the material suspended in a small volume of chloroform or chloroform:methanol (25-100 μl) and the total material spotted on a Silica TLC plate. The plates are developed in chloroform/methanol/ acetic

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acid/water (50:25:8:4, v/v) visualized by radioscanning (Bioscan) and the product, [^3H]PAF, is scraped and quantified by liquid scintillation spectroscopy. With this TLC system, the R_f value for a synthetic standard of PAF is approximately 0.33.

5

Assay (e): Methods for the evaluation of CoA-IT inhibitors on mobilization of labeled arachidonic acid in intact cells

Measurement of the effect of CoA-IT inhibitors on the transfer of [^3H]arachidonate into 1-ether phospholipids in non-stimulated inflammatory cells can be accomplished by
1 0 general application of the following specific method. Human neutrophils are isolated and resuspended ($5 \times 10^7/\text{ml}$) in Hanks Balanced Salt Solution (HBSS; Gibco). [^3H]-Arachidonic acid (100 Ci/mmol; New England Nuclear) complexed to 200 μl HBSS containing 0.25 mg/ml HSA is added to the cell suspension (1 $\mu\text{Ci}/\text{ml}$). The cells are incubated with gentle shaking at 37°C for 5 min. The reaction is
1 5 terminated by the addition of 40 ml ice-cold HBSS containing HSA (0.25 mg/ml). The cells are then removed from the supernatant fluid by centrifugation (225 g, 8 min). Unincorporated [^3H]-arachidonic acid is completely removed by two more washes of HBSS containing 0.25 mg/ml HSA. The neutrophils are resuspended in fresh buffer, exposed to various concentrations of a CoA-IT inhibitor or its vehicle and incubated without stimulation
2 0 for 2 hrs. At that time, the tubes containing the cells and buffer are extracted (Bligh & Dyer [Can. J. Biochem. Physiol. (1959) 37, 911-917]) and the phospholipid classes are separated and collected by normal phase HPLC, using a Ultrasphere Silica column (4.6 mm x 250 mm; Rainin) eluted with hexane/2-propanol/ethanol/phosphate buffer (pH 7.4)/acetic acid (490:367:100:30:0.6 v/v) for 5 min at a flow rate of 1 ml/min. The amount of
2 5 phosphate buffer in the eluting solvent is increased to 5 % over 10 min and this solvent composition is maintained until all the phospholipid classes is eluted from the column (30-40 min) (Chilton, F. H. [Methods Enzymol. (1990) 187, 157-166]). The phospholipids are converted into diradylglycerols by addition of phospholipase C, 20 units-40 units of *Bacillus cereus* phospholipase C (Sigma Type XIII) in 100 mM Tris HCl buffer (pH 7.4)
3 0 for 2.5-6 hr, then converted into 1,2-diradyl-3-acetyl-glycerols by incubation with acetic anhydride and pyridine (Chilton, F. H. [Methods Enzymol. (1990) 187, 157-166]). The phospholipid subclasses are separated by TLC in benzene/hexane/ethyl ether (50:45:4, v/v), located by image analysis (Bioscan) and the amount of radioactivity in each class is determined by zonal scraping and liquid scintillation counting.

3 5

The following is the method for assessing the ability of a compound to alter arachidonate content of cellular phospholipids, which can be generalized for any desired

cell. Specifically, mouse bone marrow-derived mast cells are removed from culture and provided with exogenous [^3H]arachidonic acid for 30 minutes. The labeled arachidonic acid which had not been incorporated into the cells is then removed by washing the cells 2 times with an albumin-containing buffer. At that point, the cells are treated with various concentrations of CoA-IT inhibitors and then placed back in culture for 24-48 hours. The phospholipids are extracted by the method of Bligh and Dyer [Can. J. Biochem. Physiol. (1959) 37, 911-917] and phospholipids separated by normal phase HPLC by the method of Chilton [Methods Enzymol. (1990)187, 157-166]. The radioactive and mole quantities of arachidonate in complex lipids are determined. At this point, cellular lipid extracts are treated with KOH (0.5 M) to remove fatty acids from complex lipids (phospholipids) and the quantities of arachidonate in these extracts can then be determined by various methods, including gas chromatography and mass spectrometry (Chilton [Methods Enzymol. (1990)187, 157-166]).

Assay (f) for measurement of stimulated eicosanoid release by human monocytes.

Human Monocyte Isolation. Leukocyte-rich leukopaks obtained from Biological Specialties (Lansdale, PA) are collected from male volunteers who are not taking anti-inflammatory drugs. Leukopaks are centrifuged (90 x g for 15 min) twice to remove the platelet-rich plasma. The cell pellet is washed by centrifugation and are resuspended in HBSS without Ca^{2+} or Mg^{2+} . Histopaque 1077 is layered under the cell suspension and centrifuged at 400 x g for 30 min to obtain the buffy coat. The interfacial buffy coat, containing monocytes and lymphocytes, is removed and saved. The buffy coat is washed twice with HBSS without Ca^{2+} or Mg^{2+} by centrifugation. The cell pellet ($4-6 \times 10^8$ cells/30mls) is resuspended in iso-osmotic media (RPMI-1640, 10% heat inactivated fetal bovine serum (FBS), 0.2 mM L-glutamine, 2.5 mM HEPES) and layered over an equal volume of 46% Percoll mixture (10 X PBS/ Percoll; 9.25 / 0.75) and 54% iso-osmotic media and centrifuged for 30 min at 1000 x g (Marshall and Roshak, Biochem. Cell Biol. 71: 331-339, 1993). The monocyte population located at the interface of the Percoll gradient is removed and washed twice in HBSS without Ca^{2+} or Mg^{2+} . This resulted in a greater than 85-90 % pure monocyte population as assessed by differential staining.

Measurement of Stimuli-Induced Eicosanoid Release. Monocytes ($5 \times 10^6/\text{ml}$) are incubated as a suspension in serum-free RPMI-1640 medium containing the vehicle DMSO (< 1%) or drug for 30 min at 27°C after which vehicle or stimuli is added for the indicated time. The stimulating agent is solubilized in DMSO and appropriate vehicle controls are

-35-

included in all experiments. The amount of stimuli is chosen from the linear portion of a concentration versus product curve usually representing 60-80% maximal stimulation over the indicated incubation time at 37°C (A23187, 1 μ M, (15 min). The reaction is terminated by reduction of pH through addition of citric acid and centrifugation (10 min, 400 x g, 4°C).

5 Cell viability is monitored before and after experiments using trypan blue exclusion. The cell-free media is decanted and stored at -70° C until analyzed. Prostaglandin E₂ and LTC₄ are directly measured in cell-free media using enzyme immunoassay (EIA) kits purchased from Caymen Chemical Co. (Ann Arbor, MI). Sample or standard dilutions are made with appropriate media and analyzed in triplicate. Results are obtained by extrapolation from a
10 standard curve prepared in the media and expressed as pg or ng/ml of sample.

In vivo assays

15

Assays (g and h) : Assay (Method) for TPA (assay g) or Arachidonic acid (assay h)-induced Inflammation

Animals:

Male Balb/c inbred mice are obtained from Charle River Breeding Laboratories
20 (Kingston, NY). Within a single experiment mice (22-25g) are age-matched. These *in vivo* experiments typically involve the use of 5-6 animals/group.

(g) TPA-induced Mouse Ear Inflammation:

Assay of Ear Edema

25 TPA (12-0-tetradecanoylphorbol 13-acetate) (Sigma Chemical Company) in acetone (4 mg/20ml) is applied to the inner and outer surfaces of the left ear of BALB/c male mice. The thickness of both ears is then measured with a dial micrometer (Mitutoyo, Japan) at both 2 and 4 hours after treatment, and the data expressed as the change in thickness (10⁻³cm) between treated and untreated ears. The application of acetone does not cause an
30 edematous response; therefore, the difference in ear thickness represents the response to the TPA. After measuring the edema, the inflamed left ears are removed and stored at -70°C until they were assayed for MPO (myeloperoxidase) activity where appropriate.

Assay of Myeloperoxidase (MPO) in Inflamed Ear Tissue:

35 On the day of the assay, partially thawed ear tissues are minced and then homogenized (10% w/v) with a Tissumizer homogenizer (Tekmar Co.) in 50 mM phosphate buffer (pH 6) containing 0.5% HTAB. The tissue homogenates are taken through three cycles of freeze-thaw, followed by brief sonication (10 sec). The method of Bradley et al.

is used with modifications as described. The appearance of a colored product from the MPO-dependent reaction of o-dianisidine (0.167 mg/ml; Sigma) and hydrogen peroxide (0.0005%; Sigma) is measured spectrophotometrically at 460 nm. Supernatant MPO activity is quantified kinetically (change in absorbance measured over 3 min, sampled at 15-sec intervals) using a Beckman DU-7 spectrophotometer and a Kinetics Analysis package (Beckman Instruments, Inc.). One unit of MPO activity is defined as that degrading one micromole of peroxide per minute at 25°C.

Statistics:

- 1 0 Statistical analysis is done using Student's "t" test. The ED₅₀ are values which cause a 50% inhibition of the inflammatory response and are calculated by regression analysis of the dose response data.

(h) Arachidonic acid induced ear inflammation assay

- 1 5 Arachidonic acid is dissolved in acetone (1mg/ear) to the left ear of BALB/c male mice. The thickness of both ears is measured with a constant pressure thickness guage 1 hour after treatment and the data expressed as the change in thickness between treated and untreated ears. Test compounds or vehicle are given at the time of AA application. The inflammatory cell infiltration is measured by MPO activity as described above in the TPA ear edema assay. After the edema measurements are made, the inflamed ears are removed and assayed for MPO activity.
- 2 0

- The anti-inflammatory effect of various standard inhibitors topically administered in the AA and TPA induced mouse ear edema model were measured for dexamethasone, scalaradial and Wyeth's compound WY 50,295 at doses of 0.2, 0.1 and 0.3 respectively. The TPA % change in edema was -50 (p<0.001), -46 (p<0.01) and -18 (ns) respectively; for AA the change was -10 (ns), -11 (ns) and -50 (p<0.001). The change in MPO for TPA model was -54 (p<0.001), -65 (p<0.001) and -36 (p<0.05) respectively; for AA it was 0 (ns), -33 (ns) and -90 (p<0.001). One hypothesis is that the AA administration to the ear overrides the need for PLA₂ mediated liberation of substrate for subsequent pro-inflammatory lipid mediator generation or AA mobilization by CoA-IT. In this case an inhibitor of an AA-metabolizing enzyme should be effective while an inhibitor of PLA₂ would be ineffective. As noted above, scalaradial and dexamethasone have little or no effect in the AA ear model at concentrations which were effective in the TPA ear model. This can be contrasted to the activity of the selective 5-LO inhibitor WY 50,295 which strongly inhibits inflammation in response to AA. The AA ear model therefore responds well to compounds that exhibit 5-LO inhibitory action and appears to be unaffected by putative
- 2 5
- 3 0
- 3 5

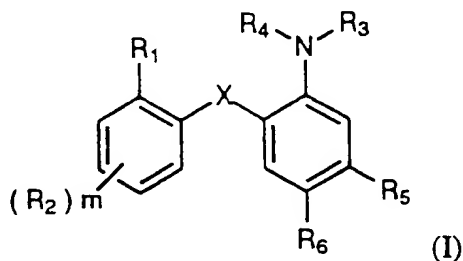
PLA₂ inhibitors. This model therefore provides a unique tool with which the contribution of the 5-LO inhibition to the in vivo anti-inflammatory activity of various compounds can be separated from LMW-PLA₂ inhibition.

5 As used herein, various abbreviations and explanations are as follows: [³H], a molecule that contains tritium atoms, a radioactive isotope; A23187, a compound that allows free entry of calcium into a cell; AA, arachidonic acid; arachidonate, arachidonic acid contained within a phospholipid; free arachidonic acid, arachidonic acid that is not contained within a phospholipid; [²H₈]arachidonic acid, the form of arachidonic acid labeled with 8
10 deuterium atoms, a stable isotope; 1-alkyl, 1-Q-alkyl; 1-alkenyl, 1-Q-alk-1'-enyl; BSA, bovine serum albumin; CoA, coenzyme A; CoA-IT, CoA-independent transacylase; DTT, dithiothreitol; EGTA, [ethylenebis(oxyethylenenitrilo)]tetra acetic acid, a calcium chelator; GPC, sn-glycero-3-phosphocholine; EDTA, a metal ion chelator; GPE, sn-glycero-3-phosphoethanolamine; GC/MS, gas chromatography and mass spectrometry; 5HETE, 5(S)-
15 hydroxyeicosa-6,8,11,14-tetraenoic acid; 15HETE, 15(S)-hydroxyeicosa-5,8,11,13-tetraenoic acid; HL-60, American Type Tissue Culture designated cell line similar to a monocyte; LTB₄, leukotriene B₄; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; lyso PAF, 1-alkyl-2-lyso-GPC, lyso platelet-activating factor; PLA₂, phospholipase A₂; PBS, phosphate buffered saline; PAF, platelet activating factor, 1-alkyl-2-acetyl-GPC; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine, PI, phosphatidylinositol; PMN, polymorphonuclear neutrophilic cell, neutrophil; PS phosphatidylserine; R_f, the distance a compound travels as a fraction of the solvent front; TLC, thin layer chromatography; U937, American Type Tissue Culture designated cell line similar to a monocyte.

25 The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its
30 fullest extent. Therefore, the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound of the formula :



5

wherein

R₁ is SO₃H or S(O)_nC₁₋₄ alkyl;

n is 0 or an integer having a value of 1 or 2;

1 0 R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;

R₃ is -C(O)R₇ or -C(S)R₇;

R₄ is hydrogen, or C₁₋₄ alkyl;

R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_mC(O)₂R₈, or (CH₂)_mOH;

m is 0 or an integer having a value of 1 or 2;

1 5 R₆ is hydrogen or halogen;

R₇ is NR₉R₁₀;

R₈ is hydrogen or C₁₋₄ alkyl;

R₉ is optionally substituted aryl, optionally substituted arylC₁₋₂ alkyl, optionally

2 0 substituted C₁₋₈ alkyl, or R₉ and R₁₀ together with the nitrogen to which they are attached form a 5 to 7 membered saturated or unsaturated ring which may optionally comprise an additional heteroatom selected from O/N or sulfur;

X is oxygen or sulfur;

provided that

2 5 a) when R₂ is 2,2-dimethyl propyl, m is 1, R₁ is SO₃H, R₅ is CF₃, and R₃ is C(O)NR₉R₁₀, and R₉ is hydrogen, than R₁₀ is other than 3,5-trimethyl phenyl ; or

b) when R₂ is 2,2-dimethyl propyl, m is 1, R₁ is SO₃H, R₅ is CF₃, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than 4-chloro-3-trifluoromethyl phenyl ;

3 0 c) when R₂ is 2,2-dimethyl propyl, m is 1, R₁ is SO₃H, R₅ is CF₃, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than 3-trifluoromethyl phenyl ;

d) when R₁ is SO₃H, R₂ is 5-methyl-4-chloro, m is 2, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 3-CF₃-4-

- chlorophenyl, 3,4-dichlorophenyl, or 2-methyl-6-chlorophenyl;
- e) when R₁ is SO₃H, R₂ is 4,6-dichlorine, m is 2, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 2-methyl-4-chlorophenyl;
- 5 f) when R₁ is SO₃H, R₂ is 4-chloro, m is 1, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 2,5-dichlorophenyl, 3,5-difluoromethyl phenyl, 2-chloro-5-CF₃-phenyl, 3-CF₃-4-chlorophenyl, 3,4-dichlorophenyl, 2,3-dichlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 3-chloro-4-(4-chlorophenoxy), 3-CF₃-6-(4-chlorophenoxy), 3-chloro-6-(4-chlorophenoxy);
- 10 g) when R₁ is SO₃H, R₂ is 4,5-dichloro, m is 2, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 2-chloro-5-CF₃-phenyl or 3-CF₃-4-chlorophenyl;
- h) when R₁ is SO₃H, R₂ is 4,5-dichloro, m is 2, R₅ is CF₃, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a 4-chloro-3-CF₃phenyl;
- 15 i) when R₁ is SO₃H, X is sulfur, R₂ is 4,5-dichloro, m is 2, R₅ is trifluoromethyl, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;
- j) when R₁ is SO₃H, X is sulfur, R₂ is 4-chloro, m is 1, R₅ is trifluoromethyl, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;
- 20 k) when R₁ is SO₃H, X is sulfur, R₂ is 4-chloro, m is 1, R₅ is chlorine, R₆ is hydrogen, and R₃ is C(O)NR₉R₁₀ and R₉ is hydrogen, than R₁₀ is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;
- 25 or a pharmaceutically acceptable salt thereof.

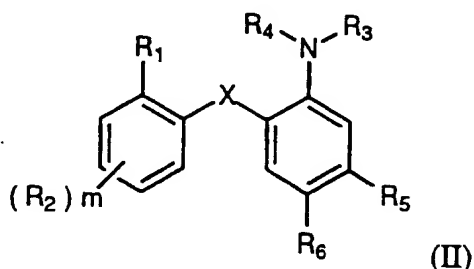
2. The compound according to Claim 1 wherein R₇ is NR₉R₁₀.
3. The compound according to Claim 2 wherein R₉ is hydrogen, and R₁₀ is hydrogen, an optionally substituted aryl, an optionally substituted arylC₁₋₂ alkyl, or optionally substituted C₁₋₈ alkyl.
- 30 4. The compound according to Claim 1 wherein X is oxygen.
- 35 5. The compound according to Claim 1 wherein R₂ is hydrogen, halogen, or optionally substituted C₁₋₈ alkyl.

6. The compound according to Claim 1 wherein R₄ is hydrogen.
7. The compound according to Claim 1 wherein R₅ is hydrogen, halogen, or CF₃.
- 5 8. The compound according to Claim 1 which is
2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
1 0 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;
2-[2-[3-(4-Methoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;
2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-
1 5 tetramethylbutyl)benzenesulfonic acid;
2-[2-[3-[3,5-bis(Trifluoromethyl)phenyl]ureido-4-methylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
2-[2-[3-[3,5-bis(trifluoromethyl)phenyl]thioureido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; and
2 0 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethyl)phenoxy]-benzenesulfonic acid.
9. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a compound according to Claim 1.
2 5
10. A method for treating an inflammatory disease or disorder in a mammal in need thereof which method comprises administering to said mammal an effective amount of a compound according to Claim 1.
- 3 0 11. The method according to Claim 10 wherein the inflammatory disease or disorder is allergic rhinitis, ischemia, reperfusion injury, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, adult respiratory distress syndrome, anaphylaxis, actinic keratosis, psoriasis, contact dermatitis, or pyresis.
- 3 5 12. The method according to Claim 11 wherein the inflammatory disease or disorder is mediated by lipid inflammatory mediators, arachidonic acid, its metabolites and/or platelet activating factor (PAF).

13. The method according to Claim 12 wherein the lipid inflammatory mediators are inhibited by the an inhibitor of the enzyme phospholipase A₂ (PLA₂) or Coenzyme A independent transacylase (CoA-IT).

5

14. A compound of the formula:



wherein

10 R₁ is SO₃H or S(O)_nC₁₋₄ alkyl;

n is 0 or an integer having a value of 1 or 2;

R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;

n is an integer having a value of 1 or 2;

R₃ is -C(O)R₇ or -C(S)R₇;

15 R₄ is hydrogen, or C₁₋₄ alkyl;

R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₈, or (CH₂)_tOH;

t is 0 or an integer having a value of 1 or 2;

R₆ is hydrogen or halogen;

R₇ is optionally substituted aryl, optionally substituted arylC₁₋₂ alkyl, or optionally

20 substituted C₁₋₈ alkyl ;

R₈ is hydrogen or C₁₋₄ alkyl;

X is oxygen or sulfur;

or a pharmaceutically acceptable salt thereof.

25 15. The compound according to Claim 14 wherein R₇ is an optionally substituted aryl or an optionally substituted aryl alkyl.

30 16. The compound according to Claim 15 wherein the aryl moiety or aryl alkyl moiety is a phenyl or benzyl which group is optionally substituted one to two times by halogen or trifluoromethyl.

17. The compound according to Claim 14 wherein X is oxygen.

18. The compound according to Claim 14 wherein R₂ is hydrogen, halogen, or optionally substituted C₁₋₈ alkyl.
- 5 19. The compound according to Claim 14 wherein R₄ is hydrogen.
20. The compound according to Claim 14 wherein R₅ is hydrogen, halogen, or CF₃.
2-[2-(Phenylmethylacetamido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
1 0 2-[2-[3,5-bis(trifluoromethyl)benzamido-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
21. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a compound according to Claim 14.
- 1 5 22. A method for treating an inflammatory disease or disorder in a mammal in need thereof which method comprises administering to said mammal an effective amount of a compound according to Claim 14.
- 2 0 23. The method according to Claim 22 wherein the inflammatory disease or disorder is allergic rhinitis, ischemia, reperfusion injury, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, adult respiratory distress syndrome, anaphylaxis, actinic keratosis, psoriasis, contact dermatitis, or pyresis.
- 2 5 24. The method according to Claim 23 wherein the inflammatory disease or disorder is mediated by lipid inflammatory mediators, arachidonic acid, its metabolites and/or platelet activating factor (PAF).
- 3 0 25. The method according to Claim 24 wherein the lipid inflammatory mediators are inhibited by the an inhibitor of the enzyme phospholipase A₂ (PLA₂) or Coenzyme A independent transacylase (CoA-IT).

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US95/06961

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 2,715,643 (MARTIN) 16 August 1955. Entire document.	1-9
X	US, A, 2,649,476 (MARTIN) 18 August 1953. Entire document.	1-9
X	US, A, 2,311,062 (MARTIN ET AL) 16 February 1943. Entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

22 AUGUST 1995

Date of mailing of the international search report

07 SEP 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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Authorized officer

NICKY CHAN

Telephone No. (703) 305-1235

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

I. Claims 1-13, drawn to urea and thiourea derivatives.

II. Claims 14-25, drawn to amido and thioamido derivatives.

The inventions listed as groups I and II do not relate to a single inventive concept under PCT Rule 13.1, because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The two groups of inventions involve different chemical functional entities that do not behave similarly in chemical and biological interactions.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEAI REPORT

Intelligence Classification No.

PCT/US95/06961

A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

A61K 31/17, 31/185, 31/19, 31/24;
C07C 275/28, 275/34, 275/40, 309/32.

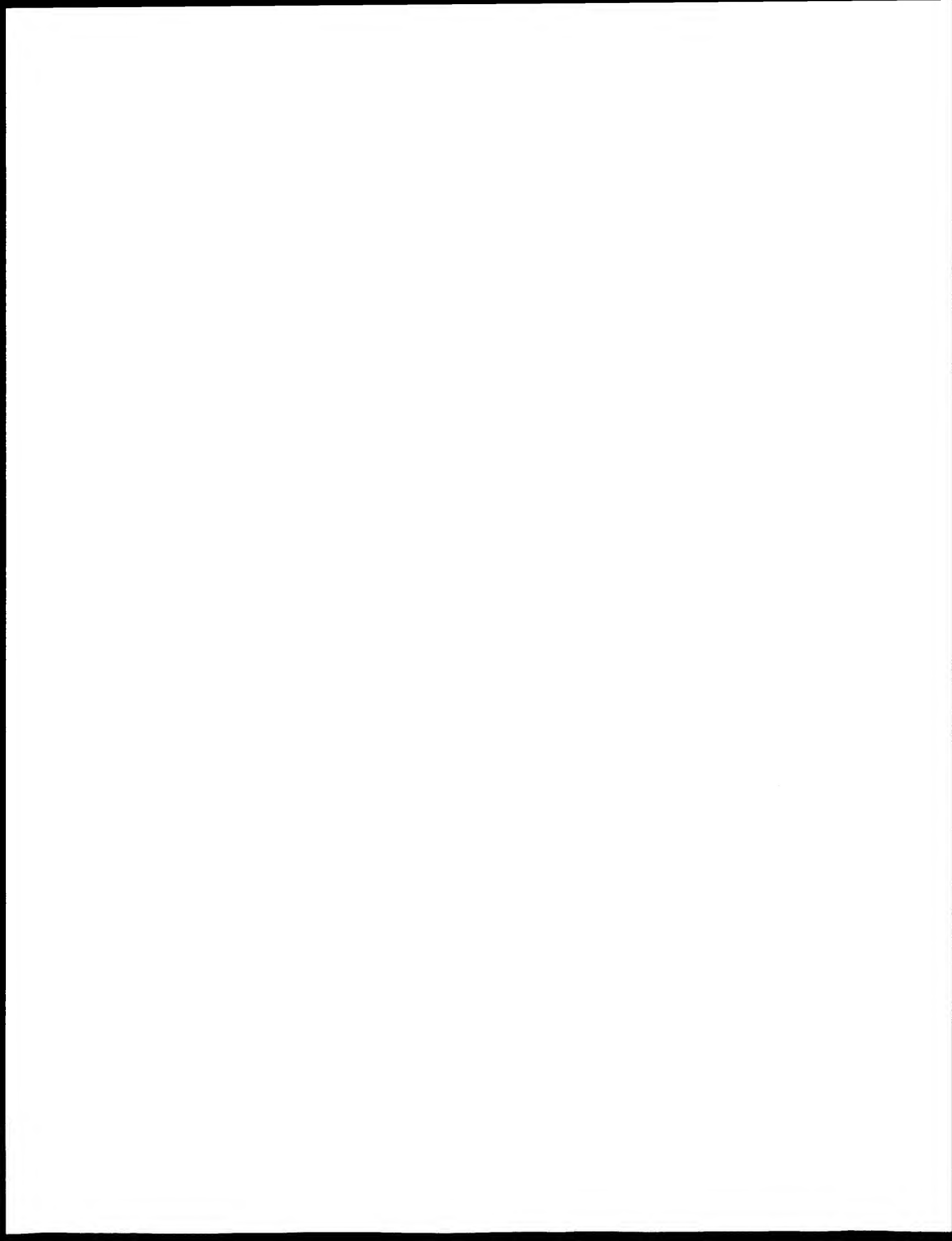
A. CLASSIFICATION OF SUBJECT MATTER: US CL :

514/598, 596, 587, 585, 576, 564, 562, 539;
560/13, 34;
562/47, 48, 430, 439;
564/26, 29, 49, 52.

B. FIELDS SEARCHED

Minimum documentation searched
Classification System: U.S.

514/598, 596, 587, 585, 576, 564, 562, 539;
560/13, 34;
562/47, 48, 430, 439;
564/26, 29, 49, 52.



INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PE1114	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/IE 00/ 00018	International filing date (day/month/year) 08/02/2000	(Earliest) Priority Date (day/month/year) 08/02/1999
Applicant UNIVERSITY COLLEGE CORK et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C335/08 C07C335/12 C07C335/16 C22B11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C22B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 07672 A (NOVONORDISK AS) 18 February 1999 (1999-02-18) page 3, line 20- page 6, line 20; compounds 20 and 170 on pages 17 and 21	1-4,7,8, 10
P,X	page 10, paragraph D --- -/--	11



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

4 April 2000

Date of mailing of the international search report

Name and mailing address of the ISA

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Arias-Sanz, J

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US ADAMS, JERRY LEROY ET AL: "Aryl antiinflammatory compounds, their preparation, and their activity" retrieved from STN Database accession no. 124:165248 XP002134113 RN: 174083-14-8 & WO 95 33458 A (SMITHKLINE BEECHAM CORP., USA) 14 December 1995 (1995-12-14) page 5, line 15 -page 7, line 2 ---	1,2,7,8
X	US 4 468 380 A (O'DOHERTY GEORGE O P ET AL) 28 August 1984 (1984-08-28) See compounds A.30 in column 2 and C.38 in column 5. See abstract ---	1,2,7,8
X	FR 1 511 325 A (CIBA) 5 April 1968 (1968-04-05) See page 1, left-hand column. Page 6, sixth compound, page 8, fifth and sixth compounds example 2 ---	1,2,7,8
X	CH 431 491 A (CIBA) 1967 the whole document example 1 ---	11
X	CH 411 449 A (CIBA) 1966 See page 1; page 2, compound 5; page 4, compounds 31 and 34. ---	1,2,7,8
X	DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US SZONYI, S. ET AL: "Perfluoroalkyl bilayer membranes prepared from saturated amphiphiles with fluorocarbon chains" retrieved from STN Database accession no. 118:256998 XP002134114 See Registry Number: 146993-88-6 & PROG. COLLOID POLYM. SCI. (1992), 89(TRENDS COLLOID INTERFACE SCI. VI), 149-55 , ---	1,3,6-8
	--- -/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US TRABELSI, H. ET AL: "Synthesis and vesicle formation of cationic surfactants based on trisubstituted perfluoroalkylated thiourea" retrieved from STN Database accession no. 118:220494 XP002134115 See Registry Number: 146993-90-0. 146993-91-1, 146993-92-2, 146993-93-3, 146993-94-4, 146993-95-5, 146993-88-6, 147011-97-0, 147011-98-1, 147011-99-2, 147012-00-8, 147012-01-9, 147012-02-0, 147012-03-1, 147012-04-2 & LANGMUIR (1993), 9(5), 1201-5 , XP000882612 figure 2; tables I-III ---</p>	1,3,6-8
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NESTOR, JOHN J., JR. ET AL: "Potent gonadotropin releasing hormone antagonists with low histamine-releasing activity" retrieved from STN Database accession no. 118:73773 XP002134116 See Registry Number: 79652-02-1 & J. MED. CHEM. (1992), 35(21), 3942-8 , ---</p>	1,3,5,7, 8
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NAKAYA, MICHIIHIKO ET AL: "Preparation of a thiadiazine derivative as insecticide and acaricide." retrieved from STN Database accession no. 117:126472 XP002134117 See Registry Number: 143413-41-6 & JP 04 077478 A (MITSUI TOATSU KAGAKU K. K., JAPAN) 11 March 1992 (1992-03-11) --- -/--</p>	1,3,5,7, 8

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NAKAYA, MICHIIHIKO ET AL: "Preparation of thiadiazine derivatives as insecticides and miticides" retrieved from STN Database accession no. 116:59409 XP002134118 See Registry Numbers: 138585-88-3 and 138585-89-4 & JP 03 215478 A (MITSUI TOATSU CHEMICALS, INC., JAPAN) 20 September 1991 (1991-09-20)</p>	1,3,5,7, 8
X	<p>--- DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NAKAYA, MICHIIHIKO ET AL: "Preparation of thiadiazine compounds as insecticides and miticides" retrieved from STN Database accession no. 116:41489 XP002134119 see Registry Number: 136491-56-0 & JP 03 197474 A (MITSUI TOATSU CHEMICALS, INC., JAPAN) 28 August 1991 (1991-08-28)</p>	1,3,5,7, 8
X	<p>--- US 5 021 412 A (NAKAYA MICHIIHIKO ET AL) 4 June 1991 (1991-06-04) See formula III in column 3 & DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US. NAKAYA, MICHIIHIKO ET AL Preparation of 2-(2-trifluoroethylimino)-3-benzyl-5-phenyl 1-1,3,5- thiadiazin-4-ones as insecticides an acaricides Database accession no. 115:232299 See Registry Numbers: 128273-53-0, 128273-57-4, 128273-58-5, 128273-64-3, 128273-72-3, 128273-73-4, 128273-74-5, 128273-75-6, 128273-77-8, 128273-78-9, 128273-79-0, 128273-80-3, 128273-81-4, 128273-84-7, 136491-56-0 --- -/--</p>	1,3,5,7, 8

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 40 16 071 A (ATOCHEM) 29 November 1990 (1990-11-29) page 4, line 30 - line 41; example 18 & DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US BOLLENS, ERIC ET AL. Preparation of [(perfluoroalkyl)ethyl]thioureas and -carbodiimides Database accession no. 114:206591 See Registry Numbers: 133600-49-4, 133600-50-7, 133600-51-8, 133600-52-9, 133600-65-4	1,3,6-8
A	--- US 4 980 071 A (SCHUSTER MICHAEL ET AL) 25 December 1990 (1990-12-25) the whole document	1-18
A	--- US 3 547 697 A (FROST JACKIE GENE ET AL) 15 December 1970 (1970-12-15) column 2 -----	1-18

INTERNATIONAL SEARCH REPORT

International Application No

Information on patent family members

PCT/E 00/00018

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9907672	A	18-02-1999	AU 8534198 A	01-03-1999
WO 9533458	A	14-12-1995	NONE	
US 4468380	A	28-08-1984	US 4526997 A	02-07-1985
			CA 1171782 A	31-07-1984
			PH 21525 A	16-11-1987
FR 1511325	A	05-04-1968	CH 490003 A	15-05-1970
			DE 1642237 A	19-05-1971
			ES 337700 A	16-06-1968
			GB 1178563 A	21-01-1970
			IL 27417 A	24-12-1970
			OA 2342 A	05-05-1970
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			AU 600170 B	02-08-1990
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			CA 1297873 A	24-03-1992
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			DE 3872634 A	13-08-1992
			EP 0308961 A	29-03-1989
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			JP 2710794 B	10-02-1998
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DE 4016071	A	29-11-1990	FR 2647112 A	23-11-1990

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EE 00/00018

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 4980071 A	25-12-1990	DE 3839222 A AT 70816 T EP 0370266 A GR 3004165 T JP 2174993 A	23-05-1990 15-01-1992 30-05-1990 31-03-1993 06-07-1990
US 3547697 A	15-12-1970	GB 1154309 A ES 357909 A JP 50015726 B	04-06-1969 01-04-1970 07-06-1975

09/17-1 967
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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference PE1114	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IE00/00018	International filing date (day/month/year) 08/02/2000	Priority date (day/month/year) 08/02/1999
International Patent Classification (IPC) or national classification and IPC C07C335/08		
Applicant UNIVERSITY COLLEGE CORK et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 06/09/2000	Date of completion of this report 09.04.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mercey, J Telephone No. +49 89 2399 8956 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IE00/00018

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-26 as originally filed

Claims, No.:

1-18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IE00/00018

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	9,10,12-18
	No:	Claims	1-8,11
Inventive step (IS)	Yes:	Claims	9,10,12-18
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

NOVELTY (Article 33(2) PCT)

- 1) The disclosures of the "X" documents in the International Search Report anticipate the subject-matter of Claims 1-8 and 11. Therefore, the subject-matter of said claims is not novel.
- 2) The specific compounds defined in Claims 9 and 10 are however not disclosed in the available prior art. Therefore the subject-matter of Claims 9 and 10 is novel (cf. however Item VI with regard to Claim 10).
- 3) Since the "X" documents in the International Search Report do not teach the use of the compounds disclosed therein for the extraction of a noble metal from a matrix, the subject-matter of Claims 12-18 is novel.

INVENTIVE STEP (Article 33(3) PCT)

D1 : US-A-4980071

In the light of D1, which discloses substituted thioureas of formula $R_1R_2N-CS-NH-COR_3$, wherein R_1-R_3 are optionally substituted alkyl, aryl or aralkyl groups, for the separation of complexly bound heavy-metal ions from reaction solutions, the problem to be solved by the present invention may be regarded as the provision of complexing agents for the extraction of noble metals.

The solution provided by Claim 12 consists of a method of extracting noble metals from a matrix using a fluorinated thiourea of formula $R^1R^3CSNR^2R^4$. Said compounds differ from those of D1 in that they do not possess a group $-COR_3$ as one of the thiourea nitrogen substituents, and in that they contain at least one fluorine atom, no fluorinated derivatives being mentioned in D1. The specific compounds *per se* of Claims 9 and 10 also differ in this manner. Since there is no suggestion in either D1, nor in any of the other cited art (no metal complexing effect being disclosed in any of the "X" documents), that the compounds of D1 may be modified in this manner and complexing agents for noble metal obtained, the method and use according to Claims 12-18, and the novel compounds *per se* of Claims 9 and 19, are considered to be inventive.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IE00/00018

Item VI

Certain documents cited

Certain published documents (Rule 70.10)

<u>Application No</u> <u>Patent No</u>	<u>Publication date</u> <u>(day/month/year)</u>	<u>Filing date</u> <u>(day/month/year)</u>	<u>Priority date (valid claim)</u> <u>(day/month/year)</u>
WO-A-9907672	18/2/99	24/7/98	5/8/97 & 11/8/97

This document discloses thiourea derivatives which fall within the scope of the claims of the present application (cf. in particular present Claim 10).

Re Item VIII

Certain observations on the international application

A double bond is missing between the C and N atoms in the isothiocyanate of Claim 11. Accordingly, R³ should not be present in said isothiocyanate, resulting in R³ always being H in the formula of Claim 1.



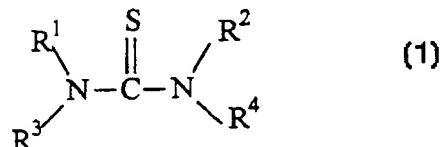
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07C 335/08, 335/12, 335/16, C22B 11/00		A1	(11) International Publication Number: WO 00/47556
			(43) International Publication Date: 17 August 2000 (17.08.00)
(21) International Application Number: PCT/IE00/00018 (22) International Filing Date: 8 February 2000 (08.02.00) (30) Priority Data: S990086 8 February 1999 (08.02.99) IE (71) Applicant (for all designated States except US): UNIVERSITY COLLEGE CORK [IE/IE]; College Road, Cork (IE). (72) Inventors; and (75) Inventors/Applicants (for US only): GLENNON, Jeremy, Denis [IE/IE]; Kileden, Nova Court, Carrigaline, County Cork (IE). HARRIS, Stephen, John [GB/IE]; 10 Broadford Crescent, Ballinteer, Dublin 16 (IE). (74) Agent: F.F. GORMAN & CO.; 54 Merrion Square, Dublin 2 (IE).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report.	

(54) Title: COMPLEXING AGENTS

(57) Abstract

A substituted thiourea having general formula (1) characterised in that each of R¹ and R² independently comprises an alkyl, alkaryl or aryl group or a substituted derivative thereof, and contains at least one fluorine atom, and in that each of R³ and R⁴ is selected from the group which consists of H, alkyl, alkaryl and aryl and substituted derivatives thereof, including fluorine-containing derivatives. A method for producing the substituted thiourea is disclosed, and a method for extracting a noble metal such as gold from a matrix by treating the matrix with the substituted thiourea is also disclosed.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

"Complexing Agents"

This invention relates to a complexing agent and in particular to new fluorinated thiourea complexing agents and fluorinated thiourea complexing agents for use in the extraction of noble metals such as gold, platinum, silver, palladium and rhodium.

Complexing agents are widely employed in the extraction and recovery of metals such as gold, platinum, silver, palladium and rhodium.

For example, gold is a soft yellow metal having a melting point of 1063°C with the highest ductility and malleability of any element. It is chemically unreactive and is not attacked by oxygen or sulphur but reacts readily with halogens or with solutions containing or generating chlorine such as "aqua regia". Its most common compounds exist in the (I) and (III) oxidation states.

15

Heretofore, the extraction of gold from ore and from other solid phases such as in solid phase extraction has been commonly carried out by using cyanide or thiourea as reagents. In the most commercially important method for gold extraction finely crushed ore is treated with sodium cyanide in the presence of oxygen to give a sodium gold cyanide complex, which is typically absorbed onto activated carbon. The sodium gold cyanide complex can be re-extracted later and reduced to the metal, (H. Schmidbaur, Interdisciplinary Science Reviews, 17 (3), 213,1992 and A. Sigel and H. Sigel in "Handbook on Metals in Clinical and Analytical Chemistry", Ed. H.G. Seiler, 1994 p388) viz:

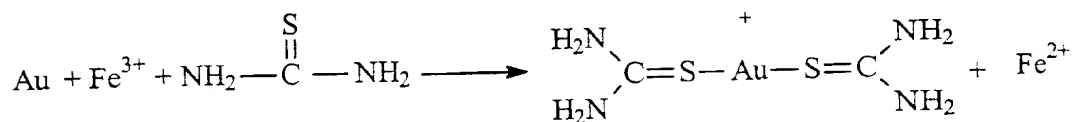
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However, treatment with sodium cyanide is environmentally unfriendly while the efficiency of the reaction can be poor and variable according to the ore type.

Accordingly, other methods of gold and silver extraction have been developed e.g. thiourea- based extraction. Thiourea-based extractions enjoy the advantages of higher leaching efficiency, rapid leaching, adaptation to a variety of refractory ores and reduced toxicity to the environment. Accordingly, thiourea is an attractive procedure for the extraction of both gold and silver.

For example, it has been demonstrated (C.K. Chen, T.N. Lung and C.C. Lung and C.C. Wan, Hydrometallurgy, 5, 207, 1980) that employing Fe^{3+} as oxidant in acid solutions resulted in leaching with thiourea which was ten times faster than leaching with sodium cyanide, viz:



However, excessive consumption of thiourea in the process has limited its industrial application.

Various attempts have been made to reduce thiourea consumption. For example, in order to reduce thiourea consumption in gold extraction it has been suggested (C.C. Kenna, Gold Bull, 24(4), 126, 1991) that the complexing of ferric ions could be utilised in reducing their oxidative power to a level where oxidation of gold still proceeded at an acceptable rate while oxidation (and consumption) of thiourea was greatly reduced.

U.S. Patent Specification No. 5126038 also discloses that alkyl hydroxamic acids or their salts may be used to improve extraction of precious metals, including gold, from sulphide ores in combination with standard sulphide ore collectors such as xanthates, substituted thioureas and the like.

5

G. Zuo and M. Muhammed, *Separation Science and Technology*, 25(13-15), 1785, 1990 also describe the synthesis and characterisation of a family of thiourea based reagents for the extraction of Au(III) and Ag(I) ions through complex formation from HCl solutions and also disclose the synthesis of several co-ordinating polymers by grafting
10 thiourea functional groups onto commercial macroporous polystyrene polymer matrices.

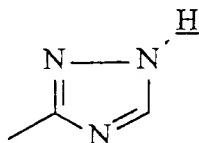
In order to avoid the use of thioureas, azacrowns have also been used to facilitate transport of $\text{NaAu}(\text{CN})_2$ into an organic phase from an aqueous phase (M. Tromp, M.
15 Burgard, M.J.F. Leroy and M. Prevost, *J. of Membrane Science*, 38, 295, 1988). In addition, Izatt et al., (R.L. Bruening, B.J. Tarbet, T.E. Krakowiak, M.L. Bruening, R.M. Izaat and J.S. Bradshaw, *Anal. Chem.*, 63(10), 1014, 1991 and R.L. Bruening, B.J. Tarbet, K.E. Krakowiak, R.M. Izatt and J.S. Bradshaw, *J. Heterocyclic Chem.*, 27 347, 1990) have developed silica gel bound thia – macrocycles which have shown high
20 selectivity for Au(III).

Supercritical fluid extraction (SFE) has developed into an attractive alternative to conventional solvent extraction to recover organic compounds from solids in particular. A useful fluid for SFE work is liquid carbon dioxide due to its moderate critical constants

($T_c = 31.1\text{ }^\circ\text{C}$, $P_c = 72.8\text{ atm}$), inertness, ease of availability, low cost and ease of final removal. However, direct extraction of metal ions by supercritical CO_2 is very inefficient due to the charge neutralisation required and weak solute-solvent interactions.

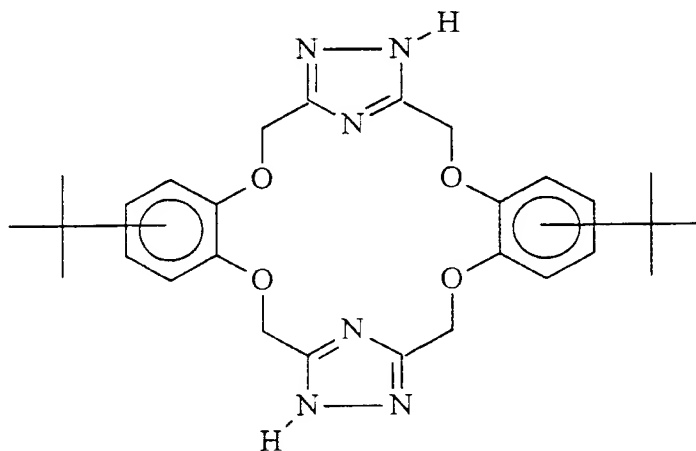
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Supercritical fluid extraction of gold has been described by S. Wang, S. Elshani and C.M. Wai, *Anal. Chem.*, 67, 919 1995 where Au(III) ions were extracted by bis-triazalocrowns from wet solid matrices using supercritical CO_2 modified with methanol. Neutral gold complexes were formed due to the presence of triazolo protons:



10

which were soluble in modified SF-CO_2 . The presence of the triazolo protons was necessary for the extraction of the metal ions to give a neutral metal ion-ligand complex:



15

and no extraction was possible without methanol modifier or water in the solid phase. Supercritical CO₂ has also been utilised (E.O. Out, Separation Science and Technology 32.6, 1107, 1997) to elute gold in the form of NaAu(CN)₂ previously adsorbed on activated charcoal employing tributylphosphate to facilitate charge
5 neutralisation. However, the presence of water in the solid phase was required for the extraction while as indicated previously the use of cyanide is undesirable for environmental and safety reasons.

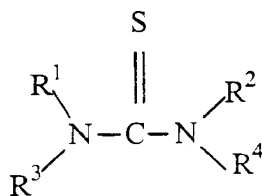
An object of the invention is to overcome the problems of the prior art.

10

A further object of the invention is to provide a complexing agent or ligand for noble metal (including gold) extraction.

A still further object of the invention is to provide a method for extracting noble metals
15 (including gold) which overcomes the problems of the prior art.

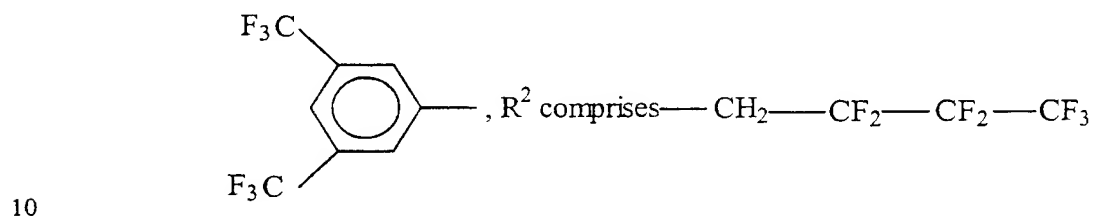
According to the invention there is provided a thiourea having the general formula:



20 wherein each of R¹ and R² independently comprises an alkyl, alkaryl or aryl group or a substituted derivative thereof, and contains at least one fluorine atom, and wherein each of R³ and R⁴ is selected from the group which consists of H, alkyl, alkaryl and

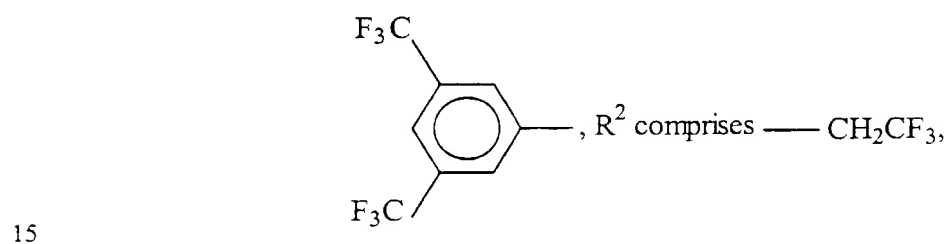
aryl, and substituted derivatives thereof, including fluorine – containing derivatives. In one aspect of the invention R^3 and R^4 are selected from the group consisting of alkyl, alkaryl and aryl, and substituted derivatives thereof including fluorine – containing derivatives. The fluorinated derivatives of the invention are extremely useful for analytical assays for the determination of gold levels, and for gold recovery, as well as for determination of platinum, silver, palladium and rhodium levels and for their recovery.

Preferably, R^1 comprises



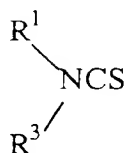
R^3 comprises H and R^4 comprises H.

Alternatively, R^1 comprises

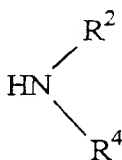


R^3 comprises H and R^4 comprises H.

The invention also extends to a method of producing a fluorinated thiourea comprising reacting a compound of general formula

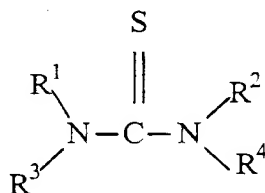


5 with a compound of general formula



where both R^1 and R^2 contain fluorine.

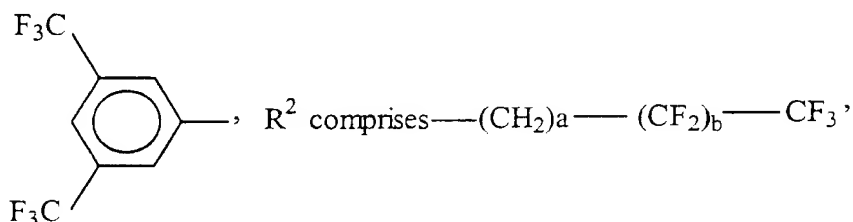
The invention also relates to a method for extracting gold from a matrix comprising
10 treating the matrix with a thiourea having the general formula



where R^1 and R^2 comprise a fluorine containing alkyl, alkaryl, aryl or substituted
derivatives thereof and R^3 and R^4 are selected from the group comprising H, alkyl, aryl
15 or substituted derivatives thereof and subjecting the matrix to supercritical fluid
extraction (SFE).

Preferably, the supercritical fluid used in the extraction comprises liquid carbon dioxide.

Preferably, R^1 comprises



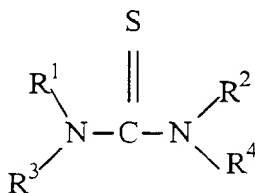
R^3 comprises H and R^4 comprises H where $a \geq 1$ and $b = 0 - 6$.

In one embodiment of the invention the gold is extracted in the presence of an oxidant.

10 Suitably, the oxidant comprises Fe (III) ions.

Advantageously, $b > 3$, i.e. $b = 4, 5$ or 6 .

The invention also extends to the use of a fluorinated thiourea of the general formula

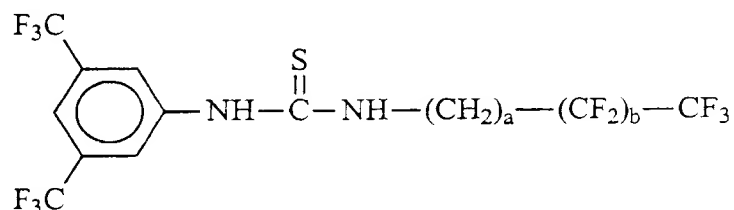


where R^1 and R^2 comprise a fluorine containing alkyl, alkaryl, aryl or substituted derivatives thereof and R^3 and R^4 are selected from the group comprising H, alkyl, aryl,

alkaryl or substituted derivatives thereof in the extraction of a noble metal, including gold, platinum, silver, palladium and rhodium from a matrix.

Surprisingly, it has been found that fluorinated thioureas of the general formula:

5



wherein $a \geq 1$ and $b = 0 - 6$

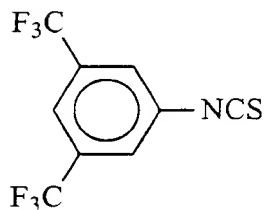
efficiently extract Au(I) and Au(III) from a solid matrix in unmodified supercritical CO₂
10 and furthermore may extract gold from a solid containing gold in its elemental form in the presence of an oxidant. Fe(III) ions are particularly suitable as oxidants. The addition of modifiers or protons is not required and extraction can be carried out using fluorinated thioureas alone.

15 In contradistinction, thiourea itself does not form a neutral complex with gold. It forms Au [SC(NH₂)₂]₂⁺.

In a preferred embodiment of the invention $b > 3$, i.e. $b = 4, 5$ or 6 .

20 The fluorinated thioureas of the invention have a high solubility in supercritical CO₂ and are extremely efficient at solubilising and carrying noble metals such as gold for the purposes of extraction, recovery, deposition or impregnation.

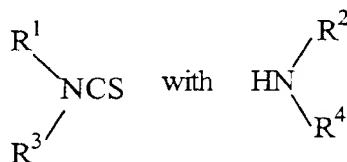
The fluorinated thioureas can be synthesised in a simple one-step process by the reaction of:



5

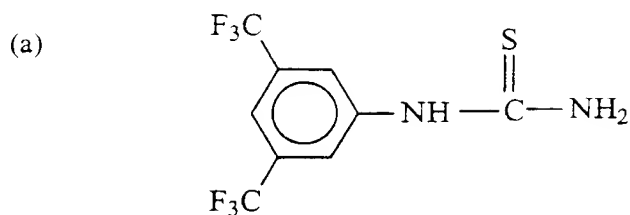
with the appropriate amine $\text{NH}_2\text{CH}_2(\text{CF}_2)_b\text{CF}_3$ where $b = 0$ or 2 to 6 in a room temperature (exothermic) reaction and recrystallisation from petroleum ether (100-120) to give colourless products in 62 to 81% yields.

10 The compositions of the invention can therefore be formed by the simple reaction of:



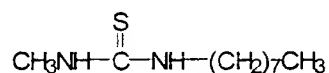
where both R^1 and R^2 contain fluorine.

15 Various embodiments of the invention will now be described by way of Example only, having regard to the following data and examples.

Comparative Examples

The aforementioned fluorinated thiourea is known from the prior art and is
5 commercially available from FLUOROCHEM.

(b)



10 In a round bottom flask 6.46g (0.05 mole) of octyl-1-amine was added to 3.65g (0.05 mole) melted methyl isothiocyanate, with stirring, under nitrogen in an ice bath. A rapid exothermic reaction ensued and the reaction was allowed to reach room temperature overnight. The colourless solid product (10.02g, 99% yield) was recrystallised from

15 Note: The oil, which settles out on cooling, solidifies on standing, yield 99%.

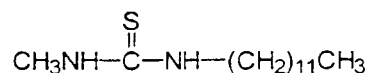
Elemental Analysis for $C_{10}H_{22}N_2S$

Calculated: C: 59.36, H: 10.96, N: 13.84, S: 15.84%

Found: C: 59.60, H 10.99, N: 13.50, S: 16.11%

(c)

20



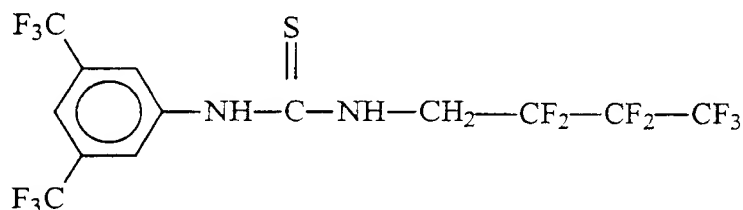
In a round bottom flask 9.27g (0.05 mole) dodecyl-1-amine was added to 3.65g (0.05 mole) of melted methyl-isothiocyanate with stirring under nitrogen. A rapid exothermic reaction ensued and the reaction mixture was allowed to reach room temperature overnight. The colourless solid product 12.45g (96% yield) was recrystallised from 100-120°C petroleum ether to give 12.27g (95% yield) of N-methyl, N'-dodecyl thiourea as white crystals.

Elemental Analysis for $C_{14}H_{30}N_2S$

Calculated: C: 65.05, H: 11.70, N: 10.84, S: 12.40%

Found: C: 64.90, H: 11.67, N: 11.10, S: 12.78%

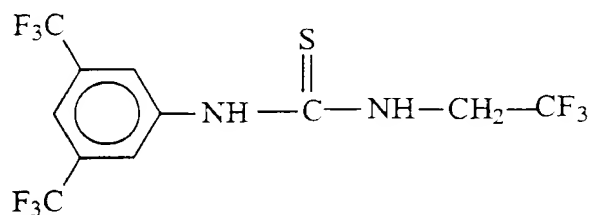
10 Example 1



To 0.518 g (0.0026mole) 1H, 1H-heptafluorobutylamine (Fluorochem (Trade Mark) Product FO4396) in a round bottom flask was added 0.705g (0.0026mole) 3,5-di(trifluoromethyl) phenylisothiocyanate (Fluorochem (Trade Mark) Product F03115B). After stirring for one minute the miscible liquids solidified to a colourless solid in an exothermic reaction. After the reaction mixture had cooled it was allowed to stand for 1 hour at room temperature and the product was recrystallised from petroleum ether (100-120) to give 0.972 g of pure product (80% yield) as colourless crystals, mp 130-132°C.

Elemental Analysis for $C_{13}H_7N_2SF_{13}$:

Calculated: C: 33.20, H: 1.50, N:5.96, Found C: 33.05, H: 1.49, N: 6.12%

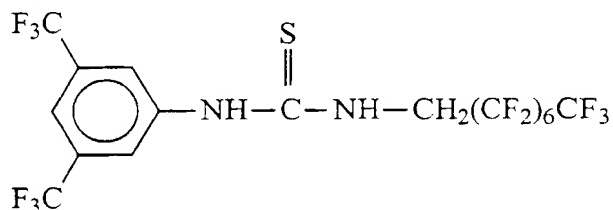
Example 2

5

To 0.705g (0.0026 mole) 3,5-di(trifluoromethyl)-phenylisothiocyanate in a round bottom flask cooled in an ice bath, with stirring under nitrogen, was added 0.281g (0.00283 mole) trifluoroethyl amine (Aldrich (Trade Mark) product 26,904-2). After a short period an exothermic reaction occurred to give a colourless solid. The reaction mixture was then allowed to warm to room temperature and was left for 1 hour under a stream of dry nitrogen to remove excess volatile amine (bp 36°C). The solid was recrystallised from petroleum ether (100-120) to give a pure product as fluffy colourless crystals 0.60g (62% yield), m.p. 133-136 °C.

Elemental Analysis for $C_{11}H_7N_2SF_9$:

15 Calculated: C: 35.64, H: 2.04, N:7.55, Found C: 35.94, H: 2.20, N: 7.73%

Example 3 (a)

To 3.28 (0.0121 mole) 3,5-di(trifluoromethyl) – phenylisothiocyanate in a round bottom flask was added 4.82g (0.0121 mole) 1H, 1H – perfluoro-octylamine Lancaster (Trade

20

Mark) product 16845 with stirring. An exothermic reaction rapidly ensued and after cooling to room temperature was allowed to remain for 2 hours. The white solid product was recrystallised from 100-120°C petroleum ether to give 6.50g pure product (81% yield) as a colorless crystalline solid.

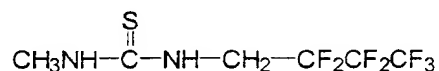
5

Elemental Analysis: $C_{17}H_7N_2SF_{21}$

Calculated: C:30.46, H:1.05, N:4.18; Found: C:30.60, H:1.16, N:4.40%

Example 3 (b)

10



In a round bottomed flask 5.92g (0.03 mole) of 1H, 1H- heptafluorobutyl amine was added to 2.18g (0.30 mole) of melted methyl-isothiocyanate with stirring under nitrogen. An exothermic reaction ensued and the reaction mixture was allowed to reach room temperature overnight. The colourless product 8.10g (100% yield) was recrystallised from 100-120°C petroleum ether to give N-methyl, N'-heptafluorobutyl thiourea, 6.91g, as a white solid in 85% yield.

Elemental Analysis for $C_6H_7N_2SF_7$

Calculated: C: 26.48, H: 2.59, N: 10.29, S: 11.78%

Found: C: 26.71, H: 2.67, N: 10.50, S: 12.25%

Example 4

The solubility of the ligand of the comparative Examples (a)-(c) in supercritical CO_2 was compared with the solubilities of the ligands of Examples 1 and 2 and 3 in supercritical CO_2 .

25

In each case, a weighed amount of the ligand of the respective comparative Example of approximately 60 mg was placed in a glass tube (2cm x 0.5cm i.d.) and plugged with glass wool at both ends. The glass tube was placed inside the extraction vessel and statically extracted for 30 minutes. The inlet valve for SF-CO₂ was then closed and the
5 outlet valve opened into a collecting solution. The loss of weight of the glass tube after SFE corresponded to the solubility of the ligand in 2.2 ml 100% SF-CO₂. The procedure was carried out at 60°C and two different pressures namely 200 and 300 atmospheres.

10 In all three cases, most of the ligand appeared to remain in the glass tube indicating poor solubility in SF-CO₂.

Solubility of the ligand of Example 1 in supercritical CO₂:

The procedure outlined above in Example 4 was repeated for the ligand of Example 1.
15 This time none of the ligand remained in the glass tube, indicating excellent solubility in SF-CO₂ at both pressures.

Solubility of the ligand of Example 2 in supercritical CO₂:

The procedure outlined above in Example 4 was repeated for the ligand of Example 2.
20 Again, none of the ligand remained in the glass tube, indicating excellent solubility in SF-CO₂ at both pressures.

Solubility of the ligand of Example 3(a) in supercritical CO₂:

The procedure outlined above in Example 4 was repeated for the ligand of Example
25 3(a).

Again, none of the ligand remained in the glass tube, indicating excellent solubility in SF-CO₂ at both pressures.

Solubility of the ligand of Example 3(b) in supercritical CO₂:

- 5 The procedure outlined above in Example 4 was repeated for the ligand of Example 3(b).

Again, none of the ligand remained in the glass tube, indicating excellent solubility in SF-CO₂ at both pressures. Larger amounts of ligand 3(b) were used and solubilities in excess of 0.7 M were thus found at both pressures.

- 10 Thus the newly synthesised thioureas were found to be highly soluble in supercritical CO₂ compared to the fluorinated and two non-fluorinated thioureas of the Comparative Examples (a)-(c).

- A number of experiments were carried out to demonstrate the extraction efficiencies of the fluorinated thioureas of the invention.
- 15

Example 5 (a) (comparative): Supercritical Fluid Extraction of Au(III) as AuCl₄⁻ using the compound of the comparative Example (a).

- 20 Gold Au(III) extraction by the ligand of the comparative Example (a) was investigated employing a BDH Gold (III) standard containing 1000 ppm Au(III) (aqueous AuCl₄⁻). Thus 60 µl of solution Au(III) containing 3.05×10^{-7} moles Au(III) was applied to a 3cm diameter filter paper. The filter paper was allowed to dry and then placed in a glass tube (2cm x 0.5cm i.d.), plugged with glass wool at both ends. 20mg of ligand of the
- 25 comparative Example (a) (in excess of over 200 fold over Au(III) level) was then placed

in the same glass tube and plugged with glass wool. The temperature of the extraction vessel was then set at 60°C and the pressure was varied as indicated in Table 1.

The extraction vessel was statically extracted for 20 minutes and then dynamically
5 extracted into a collecting solvent of 4 ml methanol for 15 minutes (0.8 ml CO₂/minute flow rate). The methanol solution was then made up to 10 ml using additional methanol. Levels of gold in solution were then determined by atomic absorption spectroscopy. The procedure was carried out at different pressures from 200-400 atm. The following extraction percentages were obtained:

TABLE 1

Pressure SF-CO ₂ (atm)	Atomic Absorption (A.U.)*	Extraction (%)
200	0.000	0
250	0.000	0
300	0.001	~ 0.0
350	0.000	0
400	0.024	6.7

* The % extraction is calculated with reference to the Atomic Absorption reading obtained for 10ml of collecting solution spiked directly with 60µl of the 1000ppm Au(III) standard. (For example for the data presented in Table 1 a standard of 60 µg/10 ml = 6ppm Au, gave an absorption value of 0.359, representing 100% extraction. Note: such recordings of standard values were carried out alongside the sample analysed on
25 the same day.)

400 atm was the only pressure of SF-CO₂ to give detectable Au(III) extraction. In all runs most of the ligand appeared to remain in the glass tube indicating poor solubility in SF-CO₂.

5

Example 5 (b) (comparative): Supercritical Fluid Extraction of Au(III) as AuCl₄⁻ using the compound of the comparative Example (b).

The procedure of Example 5(a) was repeated for Comparative Example (b) to give 3.2% gold extraction at 250 atmospheres and 2.0% at 450 atmospheres pressure.

10 Most of the ligand appeared to remain in the glass tube after all runs indicating poor solubility in SF-CO₂.

Example 5 (c) (comparative): Supercritical Fluid Extraction of Au(III) as AuCl₄⁻ using the compound of the comparative Example (c).

15 The procedure of Example 5(a) was repeated for Comparative Example (c) to give 2.6% gold extraction at 250 atmospheres and 1.3% at 450 atmospheres pressure. Most of the ligand appeared to remain in the glass tube after all runs indicating poor solubility in SF-CO₂.

20 Example 6 (comparative)

The procedure in Example 5(a) was repeated except the temperature of the extraction procedure was varied from 60-120°C, while maintaining the pressure of the extractor at 400 atm. The following results were obtained:

25

TABLE 2

Temperature (°C)	Atomic Absorption (A.U.)*	Extraction (%)
60	0.027	8.8
80	0.002	0.7
100	0.006	2.0
120	0.005	1.6

* A standard of 6ppm Au, gave an absorption value of 0.305 = 100%

The % extraction of gold remained low.

Example 7

The procedure in Example 6 was repeated with the ligand from Example 1 being used in place of the ligand of the Comparative Example (a). The following % extraction values were obtained at differing pressures for extraction of Au(III):

TABLE 3

	Pressure SF-CO ₂ (atm)	Extraction (%)
5		
	200	61.2
	250	92.7
	300	83.7
10	350	75.3
	400	78.1

Percentage extraction with the ligand of the invention was therefore excellent at 92.7% compared with the poor extraction (<10%) with the ligands of the comparative Examples.

Example 8

Reduction of Au(III) to Au(0) was accomplished by treatment with hydroxylamine hydrochloride followed by sodium hydroxide.

The procedure in Example 7 was repeated except the conditions were altered to 60°C at 250 atm. The 60µl of AuCl₄⁻ was replaced by 60µl of a well mixed even suspension of Au(0) applied to the filter paper which was allowed to dry to give a black-blue colour.

The collected methanol solutions, made up to 10 ml as before, were analysed by Atomic Absorption Spectroscopy as before, giving the following % extraction values:

TABLE 4

5	Pressure SF-CO ₂ (atm)	Atomic Absorption (A.U)*	Extraction (%)
10	200	0.001	~ 0.0
	250	0.002	~ 0.0
	300	0.0001	~ 0.0
	350	0.002	~ 0.0
	400	0.002	~ 0.0
15			

Accordingly, the ligand of Example 1 did not extract Au(0). Moreover, at the end of the runs the blue-black colour of Au(0) remained on the filter paper and no ligand remained in the tube. However, as described further below the gold could be extracted following oxidation of Au(0) to either Au(I) or Au(II).

Example 9

The procedure in Example 8 was repeated but 40mg of solid Au(0) was used in place of the Au(0) suspension deposited on the filter paper to give an identical result to Example 8.

Example 10

The procedure of Example 9 was repeated utilising 20 mg of the ligand Example 1 except 11.2 mg of solid Au(0) was used and on two occasions the Au(0) was first oxidised to Au(I) by spiking 60 µl of Fe(III) (1000 ppm stock solution) onto the solid
5 Au(0) directly and then allowed to dry. In the case where Fe(III) had been added when the collected methanol solution (as usual made up to 10 ml) was analysed by atomic absorption a large signal was obtained (0.252) and (0.253) indicating extraction of Au(I). Fe(III) was the limiting reagent as:



and therefore 60µl 1000ppm Fe(III) (1.075µmole) is equivalent to 212µl of 1000ppm Au(I) (0.2 mg Au).

15 Table 5 below outlines the results obtained following application of Fe(III):

20

25

30

35

TABLE 5

Sample	Atomic Absorption (A.U.)*	Extraction (%)
5	11.2mg Au(0) + 20mg ligand of Example 1	0.001
0		
10	11.2 mg Au(0) + 60 µl Fe(III) + 20mg ligand of Example 1	0.252
20.0		
15	11.2 mg Au(0) + 60 µl Fe(III) + 20mg ligand of Example 1	0.253
20.0		

A 212µl aliquot of 1000 pp, Au(III) standard solution gave an absorption value of 1.263
= 100%.

Accordingly, Au(0) has been successfully oxidised by Fe(III) to give Au(I) which has
been extracted with the fluorinated ligand of the invention. That only 20% of the
theoretically freed gold (by Fe) was finally detected by atomic absorption is not
surprising in view of the fact that the Fe(III) was applied as a 1000ppm aqueous nitrate
solution to the solid Au(0) on the filter paper without thorough mixing.

Nevertheless, the method can be employed successfully as a qualitative test for Au(0).

Example 11

- 5 The procedure of Example 7 was repeated employing the ligand of Example 2 in place of the ligand of Example 1 and utilising 50 μ l Au(III) standard solution in place of 60 μ l to give the following % extraction results at differing pressures of SF-CO₂ at 60°C.

TABLE 6

10

Atmosphere SF-CO ₂	Extraction (%)
-------------------------------	-------------------

15

200	41
250	51
300	22
400	14

20

Example 12

- The procedure of Example 11 was repeated except Au(III) standard was replaced by 5.7 mg solid Au(0) and extraction was determined under previously optimised
25 conditions 60°C/250 atm SF-CO₂. Table 7 summarises the results:

TABLE 7

5	Sample	Extraction (%)
10	Au(O) + ligand	~ 3

Example 13

15 Above Example 12 was repeated except that 30 μ l 1000 ppm Fe(III) standard (aqueous nitrate) was spiked onto the Au(0) prior to extraction with SF-CO₂ (250atm/60°C).

Table 8 below summarises the results obtained.

TABLE 8

20	Sample	Extraction (%)
25	Au(O) + 30 μ l Fe(III) + 20mg ligand	57
30		

The above percentage is based on the Fe(III) oxidisable quality of gold i.e. Fe(III) is the limiting reagent.

35 57% of the "freed" gold Au(I) was therefore extracted with the fluorinated ligand of the invention.

Advantages of the invention include (but are not limited to) the following:

The linear fluorinated thioureas of the invention therefore have the unexpected property
5 of extracting Au(III) in supercritical CO₂. In addition Au(I) may be extracted from Au(0)
(in its elemental state) by prior treatment with Fe(III).

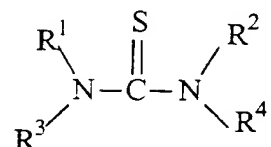
The complexing agents and extraction methods of the invention are highly efficient and
do not require the use of cyanides. In addition, the fluorinated thioureas of the
10 invention facilitate the extraction of noble metals (including gold, platinum, silver,
palladium and rhodium) without excessive thiourea consumption.

Moreover, extraction of noble metals (including gold, platinum, silver, palladium and
rhodium) using fluorinated thioureas and supercritical fluid can be effected without
15 requiring the addition of modifiers, protons and the like.

The invention is not limited to the embodiments herein described which may be varied
in construction and detail.

Claims

1. A substituted thiourea having the general formula

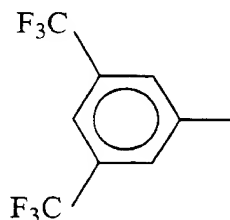


5

characterised in that each of R^1 and R^2 independently comprises an alkyl, alkaryl or aryl group or a substituted derivative thereof, and contains at least one fluorine atom, and in that each of R^3 and R^4 is selected from the group which consists of H, alkyl, alkaryl and aryl and substituted derivatives thereof, including fluorine-containing derivatives.

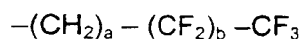
10

2. A thiourea according to Claim 1 wherein R^1 is



15

3. A thiourea according to Claim 1 or 2 wherein R^2 is



20 wherein a is an integer not less than 1 and b is a whole number from 0 to 6.

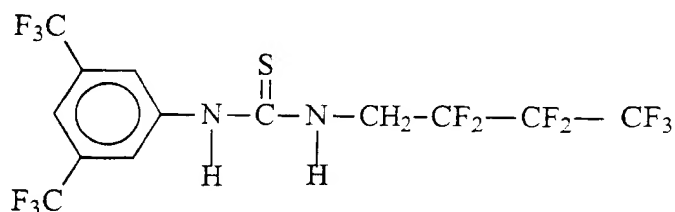
4. A thiourea according to Claim 3 wherein R^2 is $-\text{CH}_2 - \text{CF}_2 - \text{CF}_2 - \text{CF}_3$.

5. A thiourea according to Claim 3 wherein R^2 is $-\text{CH}_2 - \text{CF}_3$.

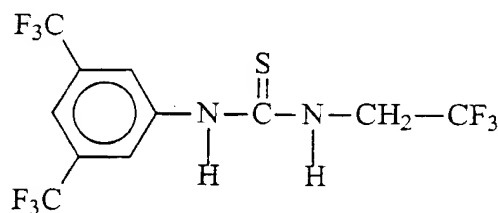
25

6. A thiourea according to Claim 3 wherein b is 4, 5 or 6.

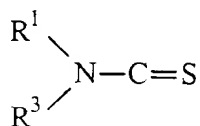
7. A thiourea according to any preceding claim wherein R^3 is H.
8. A thiourea according to any preceding claim wherein R^4 is H.
- 5 9. A thiourea according to Claims 2, 4, 7 and 8, viz. one of formula



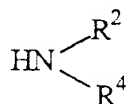
- 10 10. A thiourea according to Claims 2, 5, 7 and 8, viz. one of formula



11. A method of producing a substituted thiourea of the formula given and
 15 defined in any preceding claim, characterised by reacting a compound of general formula



with a compound of general formula



20 wherein R^1 , R^2 , R^3 and R^4 are as defined in any said claim.

12. A method of extracting a noble metal from a matrix, said method characterised by treating the matrix with a substituted thiourea according to any of Claims 1 to 10, and subjecting the thus treated matrix to supercritical fluid extraction.

5 13. A method according to Claim 12 wherein the noble metal is gold, platinum, silver, palladium or rhodium.

14. A method according to Claim 12 or 13 wherein the supercritical fluid is liquid carbon dioxide.

10

15. A method according to any of Claims 12 to 14 wherein the treatment with substituted thiourea is performed in the presence of an oxidant.

15

16. A method according to Claim 15 wherein the oxidant comprises ferric (Fe^{III}) ions.

20

17. A method according to any of Claims 12 to 16 wherein the treatment and extraction are carried out at room temperature and are followed by recrystallisation of the product from petroleum ether (100-120°).

18. Use of a thiourea according to any of Claims 1 to 10 in the extraction of gold, platinum, silver, palladium or rhodium from a matrix.

INTERNATIONAL SEARCH REPORT

Interr Application No

PCT/IE 00/00018

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C335/08 C07C335/12 C07C335/16 C22B11/00

According to international Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C22B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 07672 A (NOVONORDISK AS) 18 February 1999 (1999-02-18) page 3, line 20- page 6, line 20; compounds 20 and 170 on pages 17 and 21	1-4,7,8, 10
P,X	page 10, paragraph D --- -/--	11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

4 April 2000

Date of mailing of the international search report

14. 04. 00

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INTERNATIONAL SEARCH REPORT

Inter: Application No
PCI/IE 00/00018

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US ADAMS, JERRY LEROY ET AL: "Aryl antiinflammatory compounds, their preparation, and their activity" retrieved from STN Database accession no. 124:165248 XP002134113 RN: 174083-14-8 & WO 95 33458 A (SMITHKLINE BEECHAM CORP., USA) 14 December 1995 (1995-12-14) page 5, line 15 -page 7, line 2 ---	1,2,7,8
X	US 4 468 380 A (O'DOHERTY GEORGE O P ET AL) 28 August 1984 (1984-08-28) See compounds A.30 in column 2 and C.38 in column 5. See abstract ---	1,2,7,8
X	FR 1 511 325 A (CIBA) 5 April 1968 (1968-04-05) See page 1, left-hand column. Page 6, sixth compound, page 8, fifth and sixth compounds example 2 ---	1,2,7,8
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X	CH 411 449 A (CIBA) 1966 See page 1; page 2, compound 5; page 4, compounds 31 and 34. ---	1,2,7,8
X	DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US SZONYI, S. ET AL: "Perfluoroalkyl bilayer membranes prepared from saturated amphiphiles with fluorocarbon chains" retrieved from STN Database accession no. 118:256998 XP002134114 See Registry Number: 146993-88-6 & PROG. COLLOID POLYM. SCI. (1992), 89(TRENDS COLLOID INTERFACE SCI. VI), 149-55 , ---	1,3,6-8
	-/--	

INTERNATIONAL SEARCH REPORT

Inter Application No
PC1/IE 00/00018

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US TRABELSI, H. ET AL: "Synthesis and vesicle formation of cationic surfactants based on trisubstituted perfluoroalkylated thiourea" retrieved from STN Database accession no. 118:220494 XP002134115 See Registry Number: 146993-90-0, 146993-91-1, 146993-92-2, 146993-93-3, 146993-94-4, 146993-95-5, 146993-88-6, 147011-97-0, 147011-98-1, 147011-99-2, 147012-00-8, 147012-01-9, 147012-02-0, 147012-03-1, 147012-04-2 & LANGMUIR (1993), 9(5), 1201-5 , XP000882612 figure 2; tables I-III ---</p>	1,3,6-8
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NESTOR, JOHN J., JR. ET AL: "Potent gonadotropin releasing hormone antagonists with low histamine-releasing activity" retrieved from STN Database accession no. 118:73773 XP002134116 See Registry Number: 79652-02-1 & J. MED. CHEM. (1992), 35(21), 3942-8 , ---</p>	1,3,5,7, 8
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NAKAYA, MICHIIKO ET AL: "Preparation of a thiadiazine derivative as insecticide and acaricide." retrieved from STN Database accession no. 117:126472 XP002134117 See Registry Number: 143413-41-6 & JP 04 077478 A (MITSUI TOATSU KAGAKU K. K., JAPAN) 11 March 1992 (1992-03-11) --- -/--</p>	1,3,5,7, 8

INTERNATIONAL SEARCH REPORT

Inter Publication No

PCT/IE 00/00018

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NAKAYA, MICHIIHIKO ET AL: "Preparation of thiadiazine derivatives as insecticides and miticides" retrieved from STN Database accession no. 116:59409 XP002134118 See Registry Numbers: 138585-88-3 and 138585-89-4 & JP 03 215478 A (MITSUI TOATSU CHEMICALS, INC., JAPAN) 20 September 1991 (1991-09-20) ---</p>	1,3,5,7, 8
X	<p>DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US NAKAYA, MICHIIHIKO ET AL: "Preparation of thiadiazine compounds as insecticides and miticides" retrieved from STN Database accession no. 116:41489 XP002134119 see Registry Number: 136491-56-0 & JP 03 197474 A (MITSUI TOATSU CHEMICALS, INC., JAPAN) 28 August 1991 (1991-08-28) ---</p>	1,3,5,7, 8
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INTERNATIONAL SEARCH REPORT

Inter Application No
PCT/IE 00/00018

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter: Application No

PCT/IE 00/00018

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DE 4016071	A	29-11-1990	FR 2647112 A	23-11-1990

INTERNATIONAL SEARCH REPORT

International Classification No.

PCT/US95/06961

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 31/17, 31/185, 31/19, 31/24;
C07C 275/28, 275/34, 275/40, 309/32.

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/598, 596, 587, 585, 576, 564, 562, 539;
560/13, 34;
562/47, 48, 430, 439;
564/26, 29, 49, 52.

B. FIELDS SEARCHED

Minimum documentation searched
Classification System: U.S.

514/598, 596, 587, 585, 576, 564, 562, 539;
560/13, 34;
562/47, 48, 430, 439;
564/26, 29, 49, 52.